Atlas of Genetics and Cytogenetics in Oncology and Haematology

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11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours

Bilbao, Spain, September 6-9, 2008

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Scope

The **Atlas of Genetics and Cytogenetics in Oncology and Haematology** is a peer reviewed on-line journal in open access, devoted to genes, cytogenetics, and clinical entities in cancer, and cancer-prone diseases.
It presents structured review articles on genes, leukemias, solid tumors, cancer-prone diseases ("cards"), more traditional review articles on these and also on surrounding topics ("deep insights"), case reports in hematology, and educational items in the various related topics for students in Medicine and in Sciences.

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11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours

Bilbao, Spain, September 6-9, 2008

Meeting Report

The 11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours took place in Bilbao, Spain, on September 6 to 9, 2008.

The organizer was Luis Antonio Parada, investigator at the Center for Cooperative Research on Biosciences (CICbioGUNE) and the Scientific Committee was composed of Charles Buys, Juan Cigudosa, Paul Edwards, Lidia Larizza, Felix Mitelman, Rosa Miro, and Manfred Schwab.

The workshop received generous support from The Spanish Ministry for Education and Science, Genome Spain, Agilent Technology and MicroBeam Spain. This has allowed us to invite distinguished speakers who are leading experts in the field.

The workshop was a great success with more than 120 participants from the vast majority of European countries. There was enthusiastic participation of many researchers, reflecting the fact that the subject evidently keeps attracting many cytogeneticists and molecular geneticists, in particular many notable young scientists. Of course nothing better reflects the collective background of the participants, and all the new information they provided, than the abstracts themselves that are now available as follow.

Luis Antonio Parada

Guest Editor

CIC bioGUNE

11th EWCMGST Organizer

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Genetic changes in solid tumours

High resolution survey of homozygous deletions in cancer

GR Bignell, CD Greenman, AP Butler, A Futreal, MR Stratton

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Published in Atlas Journal in October 2008

Abstract

Oral presentation

Homozygous deletions are often seen in cancer as a means of inactivating tumour suppressor genes. However, such deletions can also result from genome instability and as such probably represent passenger events that do not contribute to carcinogenesis. These passenger deletions are often associated with known regions of genomic fragility (fragile sites). We have analysed approximately 800 cancer cell lines together with 466 normal DNAs using one of the latest high density SNP arrays (Affymetrix Genome-Wide Human SNP Array 6.0 containing over 1.8 million loci). This screen provides integrated analysis of copy number and genotyping information and is probably the largest analysis of its type performed to date. Over 10000 homozygous deletions were identified within the set of cancer cell lines. Of these >3400 deletions were putatively somatic. These somatic homozygous deletions collectively accounted for over 552Mb of genomic DNA (>18% of the genome). There were approximately 300 clusters of two or more overlapping homozygous deletions which accounted for ~2200 deletions. 14 clusters include known tumour suppressor genes and account for ~450 homozygous deletions, while ~300 deletions are within the defined footprint of the well mapped common fragile sites. Analysing the deletion patterns seen over known loci provides a means to classify previously unknown homozygous deletion clusters. There is evidence that a substantial proportion of these may be regions of cancer associated fragility, although a minority may be novel tumour suppressor genes.

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Genetic changes in solid tumours

Genomic profiling of breast cancers

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Abstract *Oral presentation*

Breast cancer (BC) is a complex, heterogeneous disease at the molecular level. Accumulation and combination of genetic and epigenetic alterations cause tumorigenesis, genetic instability, and acquisition of an increasingly invasive and resistant phenotype. High-throughput molecular analyses provide an unprecedented opportunity for resolving BC heterogeneity and identifying new classes biologically and clinically relevant. We recently reported the integrated genomic profiling of basal and luminal breast cancers (Adelaide et al, Cancer Res., 2007) by applying combined high resolution genomic analyses (244K aCGH [Agilent Technologies] + U133 Plus 2.0 human [Affymetrix]). The results support the existence of specific oncogenic pathways in basal and luminal BCs, involving several potential oncogenes and tumor suppressor genes (TSG). In basal tumors, 73 candidate oncogenes were identified in chromosome regions 1q21-23, 10p14, and 12p13, and 28 candidate TSG in regions 4q32-34 and 5q11-23. In luminal BCs, 33 potential oncogenes were identified in 1q21-23, 8p12-q21 11q13 and 16p12-13 and 61 candidate TSG in 16q12-13, 16q22-24 and 17p13. HORMAD1 ($p=6.5\ 10^{-5}$) and ZNF703 ($p=7\ 10^{-4}$) were the most significant basal and luminal potential oncogenes, respectively. Using the same strategy, we have now extended our study to various molecular subtypes and other types of BC associated with poor evolution and aggressiveness including inflammatory BC and young women BCs. The integrated genomic profiles were established to facilitate the search for specific molecular signatures and new cancer-associated genes. We hope that this analysis of the different aggressive forms of BCs will provide a better understanding of mammary carcinogenesis to help in the development of appropriate treatments.

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Genetic changes in solid tumours

Chromosome translocations in breast cancer

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Abstract

Oral presentation

Relatively little is known about chromosome translocations in the common epithelial cancers such as breast cancer, in spite of the central role played by translocations and consequent gene fusions in haematopoietic cancers.

We present a comprehensive analysis by array painting of the chromosome translocations of breast cancer cell lines HCC1806, HCC1187 and ZR-75-30. In array painting, chromosomes are isolated by flow cytometry, amplified and hybridized to DNA microarrays. A total of 200 breakpoints were identified and all were mapped to 1Mb resolution on BAC arrays, then 40 selected breakpoints, including all balanced breakpoints, were further mapped on tiling-path BAC arrays or to around 2kb resolution using oligonucleotide arrays. Many more of the translocations were balanced than expected, either reciprocal (8 in total) or balanced for at least one participating chromosome (19 paired breakpoints). Many breakpoints were at genes that are plausible targets of oncogenic translocation, including *CTCF* and *P300*. Two gene fusions were also demonstrated, *TAX1BP1-AHCY* and *RIF1-PKD1L1*. A preliminary screen of paraffin sections of breast tumours revealed breaks in several genes, including *PKD1L1*, so the rearrangements are not confined to cell lines.

Our data establishes that array painting is a very effective way to map substantial numbers of translocation breakpoints and supports the emerging view that chromosome rearrangements that fuse, activate or otherwise alter genes at their breakpoints may play an important role in common epithelial cancers.

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Genetic changes in solid tumours

Patterns of genomic instability associated with cell cycle and DNA repair in EWING Sarcomas: Gene expression and a-CGH profiling

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Abstract

Oral presentation

Ewing's sarcoma (ES) is characterized by specific chromosome translocations, the most common being t(11;22)(q24;q12). Additionally, other type of genetic abnormalities may occur and be relevant for explaining the variable tumour biology and clinical outcome. We have carried out a high-resolution arrayCGH and expression profiling on 25 ES tumour samples to characterize the DNA copy number aberrations (CNA) occurring in these tumours and determine their association with gene-expression profiles and clinical outcome. CNA were observed in 84% of the cases. We observed a median number of three aberrations per case. Besides numerical chromosome changes, smaller aberrations were found and defined at chromosomes 5p, 7q and 9p. All CNA were compiled to define the smallest overlapping regions of imbalance (SORI). A total of 35 SORI were delimited. Bioinformatics analyses were conducted to identify subgroups according to the pattern of genomic instability. Unsupervised and supervised clustering analysis (using SORI as variables) segregated the tumours in two distinct groups: one genomically stable (3 CNA). The genomic unstable group showed a statistically significant shorter overall survival and was more refractory to chemotherapy. Expression profile analysis revealed significant differences between both groups. Genes related with chromosome segregation, DNA repair pathways and cell-cycle control were upregulated in the genomically unstable group. This report elucidates, for the first time, data about genomic instability in ES, based on CNA and expression profiling, and shows that a genomically unstable group of Ewing's tumours is correlated with a significant poor prognosis.

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Genetic changes in solid tumours

Are ER+PR+ and ER+PR- breast tumours genetically different? An array CGH study

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Abstract

Oral presentation

Estrogen receptor (ER) is an accepted predictor of response to endocrine therapy. More than 20% of ER+ breast cancers express progesterone receptor (PR). Clinical observations have indicated that ER+/PR-breast cancers could present a different pattern of hormone sensitivity than ER+/PR+ ones. Furthermore, ER+/PR-tumors are more likely to have and aggressive phenotype and it could be a link between their progesterone negativity and an hypereactive growth factor signaling.
The aim of this study is to investigate the pattern of DNA copy number aberrations (CNAs) of these two subtypes of breast carcinoma, establish the smallest overlapping regions of imbalance (SORIs), and try to identify differences between them using a high-resolution array CGH (a-CGH). The agilent 44K oligonucleotide a-CGH has been applied to 25 ER+/PR+ and 25 ER+/PR-ductal infiltrating carcinomas (DICs) of the breast (histological grades I, II, and III, negative ERBB2 status). Data analysis and chromosome segmentation was performed with the InSilicoArray CCGH software. For each sample, FISH was used to validate and to define cut-off values for gains and losses. Preliminary results show: The total altered genome was 20,3% in ER+/PR+ and 31,7% in ER+/PR-. The % of gained genome was 9,4 in ER+/PR+ and 16,3 in ER+/PR-tumors. The % of lost genome was 10,9 in ER+/PR+ and 15,3 in ER+/PR-tumors. The most frequently gained chromosomes (chrs.) in ER+/PR-tumors were 1, 16, 8 and 11, and in ER+/PR-tumors were 1, 17, 11, 8 and 20. The most frequently lost chrs. in ER+/PR+ tumors were 16, 1, 6 and 11, and in ER+/PR-tumors were 17, 22, 16, 11, 1 and 8. Considering the most frequently altered chrs., we found some SORIs in common between the two subtypes of breast tumors: gains of 1q25.2-q31.3, 1q32.1, 8q24.3 and 11q13.3; also losses of 1p21.1, 8pter-p21.2, 11q14.2, 11q14.3-qter, and 16q13-qter. Despite their similar CNAs, we found that the ER+/PR-tumors showed some

frequently altered regions not present in the ER+/PR+ tumors, related to the chrs. 1, 8, 16, 17, 20 and 22. Chrs. 17 and 22 are the most differently altered in the ER+/PR-tumors compared to the ER+/PR+ ones, specifically gain of 17p13.3-p12 and loss of 22q11.23-qter. In our knowledge, this is one of the first study focused on the genetic comparison of ER+/PR+ and ER+/PR-breast tumors by aCGH technology. In a global analysis of the 41 DICs, CNAs were consistent with the results previously reported on CGH and aCGH studies. Taking into account our preliminary results, ER+/PR-tumors present a higher chromosomal instability and have a different genetic profile compared to the ER+/PR+ ones.

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Genetic changes in solid tumours

Genomic aberrations associated with poor survival in Malignant Peripheral Nerve Sheath Tumors

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Abstract

Oral presentation

Background : Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are rare neoplasias associated with a poor clinical outcome, for which few treatment options exist. Tumors may arise sporadically, but commonly develop in patients with the hereditary condition neurofibromatosis type1 (NF1). Due to the limited number of reported cases and the genomic complexity often observed, it is unclear which genetic aberrations are contributing to the initiation, progression and clinical aggressiveness, and whether MPNST etiology is similar in patients with and without NF1.

Material and methods : As part of a long-standing international collaboration involving Norway, Sweden and the Netherlands, a series of fresh-frozen material from 48 MPNSTs and 10 neurofibromas from 51 patients with (n=31) and without (n=20) NF1 history was obtained. Chromosomal and array-based comparative genomic hybridization (aCGH) were performed to assess DNA copy number changes, and genome-wide expression data for a subset of samples (n=30) was later integrated to evaluate candidate target genes within regions recurrently affected by copy number aberrations.

Results : Forty-four MPNSTs (92%) displayed copy number changes, whereas no aberrations were found in the nine neurofibromas. A small deletion at 9p was identified as the sole alteration in a plexiform neurofibroma. Most tumors presented complex profiles with a median of 16 aberrations per sample. Gains at 17q (69%), 8q (65%) and 7p (56%), and losses at 9p (46%), 11q (46%) and 17p (42%) are the most common. Several homozygous deletion could also be pinpointed, in particular 9p21 loss seen in five out of 12 samples by aCGH. Expression data confirmed significant differences in mRNA levels of several candidate genes, such as the lower expression of CDKN2A and CDKN2B at 9p21 or the very high levels of BIRC5 at 17q25. Interestingly, no significant differences were found in the genomic profiles of sporadic versus NF1-related MPNSTs. Several genomic changes showed prognostic significance independently of clinical variables or patient group. In particular, patients whose tumors displayed concurrent gains at chromosomal regions 7p and 17q, and losses at 9p, displayed a significantly worse prognosis (p=0.00008).

Conclusions: Most MPNSTs display complex profiles that make it difficult to pinpoint primary genetic events, as virtually all chromosomes show recurrent gains and/or losses of genomic material. No differences could be found in the genetic profiles of sporadic tumors as compared to those from patients with NF1. However, the simultaneous occurrence of specific genetic aberrations (gains of 7p and 17q, and loss of 9p) was strongly associated with an aggressive clinical course independently of patient group or clinical variables. Putative targets in these regions are CDKN2A (9p) and BIRC5 (17q), confirmed to be differentially expressed in MPNSTs when compared to their benign counterparts.

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Genetic changes in solid tumours

Cytogenetic and molecular cytogenetic findings in lipoblastomas

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Abstract

Poster presentation

Lipoblastoma is a benign lipomatous tumor, which arises from embryonic adipose tissue and occurs primarily in children younger than three years of age. Microscopically, it consists of small irregular lobules of adipocytes at different maturation stages separated by connective tissue septa and primitive mesenchymal areas with a loose myxoid matrix.

Here, we present a review of available cytogenetic data and the karyotypes of ten new cases of lipoblastoma, of which seven could be further studied by fluorescence in situ hybridization (FISH) concerning the involvement of the PLAG1 gene. All seven tumors with clonal aberrations harbored breakpoints in 8q11-13, in agreement with literature data; including previously published cases, 33 of 40 (82%) lipoblastomas had rearrangement of the 8q11-13 region. These rearrangements target the PLAG1 gene, which becomes up-regulated through promoter swapping. FISH revealed that five of seven cases in our series had a rearrangement of the PLAG1 gene. Occasionally, there can be difficulties in distinguishing a lipoblastoma from a conventional lipoma or a myxoid liposarcoma. As rearrangements of 8q11-13 have only been reported in 3% of conventional lipomas and never in myxoid liposarcomas, cytogenetic analysis or FISH for the PLAG1 gene can provide useful differential diagnostic information.

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Genetic changes in solid tumours

Characterization of NCI-H69 and NCI-H69AR Small Cell Lung Cancer (SCLC) cell lines by Spectral Karyotype (SKY)

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Abstract

Poster presentation

Small cell lung cancer (SCLC) represents about 15% of all lung cancers, is invariably associated with cigarette smoking and distant metastases are often present at diagnosis. SCLC shows an excellent sensitivity to the chemotherapy at the beginning of the treatment, but develops resistance after few months. Research cellular models in vitro to study SCLC are based on assays using sensitive and resistant to chemotherapy cell lines. NCI-H69 (sensitive) and NCI-H69AR (resistant) cell lines, purchased from ATCC (American Type Culture Collection) are used. NCI-H69 cell line was originally obtained from an untreated male patient diagnosed as SCLC. The NCI-H69AR was derived from the first one and shows resistance to topoisomerase II alpha inhibitors (doxorubicin). The G-banding karyotype of H69 parental cell line was previously described (Whang-Peng et al, 1982), showing several chromosome aberrations that include del(11)(q23), der(19)t(9;19), del(17)(p12) and dmins containing N-MYC (2p24.1) amplification. No hsr-bearing chromosomes were identified in parental H69 cell line. In addition, unbalanced t(5;16) was described (Slovak et al, 1991) by G-banding analysis. The analysis of NCI-H69AR revealed substantial karyotypic changes, including der(16) chromosome, hsr regions and dmins presented 16p13.1 amplification, which locates multi-drug resistance gene (MRP gene). Multi-color FISH technique (Spectral Karyotype-SKY) in combination with G-banding was used in order to screen cytogenetic aberrations of H69 and H69AR cell lines to find marker chromosomes responsible of sensitivity to topoIIa inhibitors. The current SKY protocol has been used with minor modifications to improve the spectral image.
We have analysed a minimum of ten metaphases per karyotype and they have been described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2005). The SKY of H69 cell line revealed translocations not previously defined by conventional cytogenetics [del(5)(q13), add(12)(p?), del(15)(q22qter), add(18)(q?), der(19)t(12;13;19)(?;?;q12), der(20)t(1;20)(q21;p13), der(20)t(3;20)(?;q11) and der(22)t(12;22)(p11;q11)]. The analysis of H69AR cell line showed several chromosome translocations (25~30) that were difficult to define by G-banding, and the SKY technique confirmed structural abnormalities not observed in H69 karyotype [der(1)t(1;12)(p12;q11), der(3)t(3;4)(p21;?), der(4)t(3;4)(?;p15), der(5)t(5;15)(?;?), der(7)t(1;7)(q25;q22), der(7)t(7;14)(p22;?), der(9)t(9;21)(q34;?), der(10)t(4;10)(q21;p11)x2, der(13)t(13;18)(q?;q?),der(15)t(X;15)(q11;p11),der(16)t(3;16;18;5;18),der(19)t(9;19)(q13;q12) and

der(21)t(12;21)]. Of interest, a der(16) with a complex translocation t(3;16;18;5;18) showed a hsr region with 18q amplification, and a hsr in der(13) with 18q amplification was observed. Whole chromosome painting probes (WCP) were used to confirm the complex translocation t(12;13;19) in the H69 and the structural abnormalities detected by SKY from the H69ARThe application of SKY technique provides a useful complementary technique to routine conventional cytogenetics for the accurate characterisation of SCLC cell lines. This technique allows defining new chromosomal aberrations not well defined previously, in order to set rules for future studies. The study of chromosomal aberrations in other resistant cell lines like H69AR could help to find marker regions of chemoresistance.

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Genetic changes in solid tumours

Identification of novel oncogene candidates present in the highly amplified region 22q11-12 in laryngeal cancer cell lines - preliminary results

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Abstract

Poster presentation

The diverse group of head and neck cancers includes laryngeal squamous cell carcinomas (LSCC). In the European Union the average, age adjusted incidence for this cancer is 9.1 / 100 000 for men and 0.6 / 100 000 for women. LSCC is a major health issue in European countries because the average 5-year survival equals only 62%. Amplification of oncogenes is a common event in human cancers, thus the present study was focused on an identification of the copy number amplifications in LSCC cancer cell lines to delineate novel oncogenes. We tested three cell lines (UT-SCC 11,22,34) derived from squamous cell carcinoma of the larynx at the University of Turku with array-CGH technique (Agilent -Human Genome CGH 44A), that spans the human genome with an average spatial resolution of approximately 75 kb. The obtained copy number profiles showed several copy number changes including in UT-SCC 11 cell line the highly amplified 22q11-12 region harbouring the putative oncogene: CRKL. CRKL (v-CRK avian sarcoma virus CT10-homolog-like) has been shown to activate the RAS and JUN kinase signalling pathways and transform fibroblasts in a RAS-dependent fashion. To determine possible amplification of CRKL gene in nine further cell lines (UT-SCC 8,18,19A,23,29,35,38,42,50) and three LSCC cell lines already tested by array-CGH, we applied real-time quantitative PCR both with SYBR Green I and FRET hydrolysis probes. The beta-2-microglobulin (B2M) gene was used as a reference; neither amplification nor deletions of B2M DNA were observed. The real-time analysis showed a CRKL gene amplification in 3 of 12 analyzed cell lines. We identified a gain of 5 copies CRKL gene over the ploidy level in UT-SCC 11 cell line as compared to the reference B2M gene and a gain of one CRKL copy in the UT-SCC 35 and UT-SCC 18 cell lines. The next step on the way is to check an expression of mRNA for CRKL gene. In any case, the obtained results are strongly indicative for an oncogenic character of CRKL in LSCC.

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Genetic changes in solid tumours

Array-CGH identifies tumor suppressor gene loci in laryngeal cancer cell lines

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Abstract

Poster presentation

Historically, the majority of classical tumor suppressor genes (TSG) like *CDKN2A* or *RB1* were identified by the delineation of bi-allelic losses called homozygous deletions. To identify small, hitherto undetected homozygous deletions in laryngeal squamous cell carcinoma cell lines (LSCC) and to unravel novel putative tumor suppressor genes three LSCC cell lines were screened with the high resolution array Comparative Genomic Hybridization (array-CGH) technique (Agilent -Human Genome CGH 44B). Altogether, 31 candidate regions for homozygous deletions were identified by array-CGH. Out of these, 12 regions overlapped with known polymorphic sites delineated in the Database of Genomic Variants and Human Structural Variation Database and thus were excluded from the analysis. The remaining 19 candidate regions were further tested by multiplex PCR and 5 regions were verified as homozygous deletions. Among others, these homozygous deletions affected the apoptosis inducing *STK17A* gene in one cell line and the tumor suppressor *CDKN2A* in two cell lines. To assess the frequency of the identified deletions in a larger panel of samples we investigated the affected sites in 9 additional LSCC cell lines. In 5 of the 9 cell lines the *CDKN2A* gene was found homozygously lost. Thus, *CDKN2A* was homozygously deleted in a total of 7 of 12 analyzed cell lines. No other recurrent homozygous deletions were found. Summing up, in this study, we show homozygous deletions to be a frequent mechanism of *CDKN2A* inactivation in laryngeal cancer cell lines. Moreover, we present several other genes, including the putative tumor suppressor *STK17A*, which may be inactivated by homozygous deletions and thus, potentially implicated in laryngeal squamous cell carcinoma development.

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Genetic changes in solid tumours

Narrowing the breakpoint in deletion del(8)(q12.1q22.1) detected in cell lines derived from larynx cancer

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Abstract

Poster presentation

The head and neck squamous cell carcinomas (HNSCC) are characterized by a huge variety of numerical and structural chromosome aberrations. At this stage it is a major difficulty to categorize chromosome aberrations and to select those attributed to HNSCC progression. Ten cell lines derived from laryngeal cancer were analyzed by GTG banding and comparative genomic hybridization (CGH). The number of chromosomes ranged from near-diploid to near-tetraploid but most frequently a near-triploid chromosome content was found. In the studied cell lines the following alterations were established as the most frequent: amplification of 11q13, deletions of 9p21-212, 18q, 3p12-24, gains of 3q, loss of 13q, 14q, loss or rearrangement of chromosome Y. Less frequent were the gains of 1q, 5p, 7p, 8q, 20q and losses of 8p, 7q22-qter, 22, 21q11-q21. The interstitial deletion of long arm of chromosome 8 was established by GTG in three cell lines. The aim of this study was focused on determine if any gene is damaged by this interstitial deletion. In UT-SCC-11 cell line the latter deletion was confirmed by array CGH. A further narrowing of the breakpoint by FISH using clones (BACs and fosmids) points to the region where the PDGP gene (plasma glutamate carboxypeptidase) is located. This gene can be affected by the identified 8q chromosome interstitial deletion. An involvement of this gene in prostate cancer progression was already suggested. The results of our study seem to suggest its involvement into laryngeal squamous cell carcinoma as well. We also hypothesize that because of different anatomical locations and histological types of prostate cancer and HNSCC a function of PDGP gene in carcinogenesis is not restricted to a particular cancer type or location.

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A papillary thyroid tumor of the follicular variant harboring *RET/PTC* and *PAX8/PPARg* gene fusion in different clones

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Abstract

Poster presentation

Thyroid cancer is the most common endocrine malignancy and accounts for the majority of endocrine cancer related deaths each year. Recent molecular studies have described several abnormalities associated with the pathogenesis and the progression of thyroid lesions, leading to a consistent progress in the understanding of the biology of this tumor. In particular, several chromosomal and molecular mutations have been well-identified in the two commonest types of differentiated thyroid carcinoma, papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) (1). Activating, mutually exclusive, mutations of the *BRAF, RET*, or *RAS* genes are found in up to 70% of PTC (with almost all *RAS* mutations in the PTC follicular variant, PTC-FV), whereas point mutations of the *RAS* genes and *PAX8-PPARg*. rearrangement are the most frequent genetic alterations in FTC. Due to their almost exclusive association, these molecular changes are making their way into use for clinical diagnostic testing, by RT-PCR and direct sequencing. Understanding the efficacy level of these tools may be important. An aggressive thyroid tumor in a 50 years old man was diagnosed as an invasive PTC-FV, i.e. a PTC showing both areas with papillary thyroid carcinoma type nuclear changes (PTC-NC), and areas with follicular architectural features. RT-PCR in microdissected areas with PTC-NC showed *RET/PTC3* activation (2). Cultured cells from the excised tumor showed normal male karyotype. Nuclei from touch preparations subjected to fluorescence in situ hybridization with DNA probes on purpose, revealed two separate clones: one with *RET* gene disruption and the other with *PAX8-PPARg* gene fusion. Our results demonstrate that genetic changes usually associated with different types of thyroid carcinoma, may coexist inside the same tumor lesion, and are confined to different cell clones. Since *RET/PTC* and *PAX8-PPARg* are usually associated with diverse types of differentiated thyroid lesions, the unexpected finding of the two changes in the same nodule may be explained by the histological mixed morphology of the lesion, possibly driven by the dissimilar genetic background developed in different cells.

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Genetic changes in solid tumours

Chromosome arm 8p shows complex genomic changes in bladder cancer

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Abstract

Poster presentation

Loss of chromosome arm 8p in bladder cancer has been shown by loss of heterozygosity (LOH) studies, array comparative genomic hybridisation (array CGH) and cytogenetics. 8p loss is associated with invasion and poor prognosis, but the target gene(s) remain elusive.

We carried out array CGH on 40 bladder cancer cell lines and 173 unselected bladder tumours of mixed stage & grade. Selected samples were analysed in more detail using CGH arrays with tiling path density for proximal 8p. Any rearrangements on 8p in the cell lines have been further explored using metaphase FISH, to confirm copy number changes and show the nature of the chromosome changes involved. The cell lines have also been studied for loss or retention of heterozygosity, and for levels of expression of selected genes. We found 8p loss or rearrangement in 87% of cell lines and 36% of tumours. Three of the cell lines and 11 of the tumours (7.5% and 6.3% respectively) also showed genomic amplification in proximal 8p. There appear to be several regions of amplification, which overlap with amplification regions reported in breast tumours. Cell lines and tumours showed similar profiles. Breakpoints were distributed throughout 8p and no major breakpoint cluster regions were found. Some of the cytogenetic changes identified in our cell lines were shown to be very complex. We found more homozygosity on 8p than would be expected by chance. Some regions of homozygosity were reflected in genomic copy number loss and some were not.

In summary, 8p changes in bladder cancer are complex, with apparently more than one target of loss and more than one target of gain. Further studies may elucidate the roles of these separate targeted regions.

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Genetic changes in solid tumours

Geographic heterogeneity of chromosome copy number changes in breast carcinomas

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Abstract

Poster presentation

Breast cancer is the most common malignancy in Western women and is a particularly heterogeneous disease. Our goal was to determine if there is geographic heterogeneity between Portuguese and Danish patients regarding chromosome copy number changes present in their respective breast carcinomas. We analysed 137 breast carcinomas from Portuguese patients by comparative genomic hybridization (CGH) and compared the findings with those previously reported by us on 129 Danish breast cancer patients. In order to assess heterogeneity of the most relevant genetic alterations, we evaluated the most common 11 chromosome changes in both series, namely 1q+, 8p+, 8p-, 8q+ 11q-, 13q-, 16p-, 16q+, 17p-, 17q+, and 20q+. Fisher's exact test was used to compare frequencies of genomic imbalances. Furthermore, chromosomal copy number changes were subjected to unsupervised hierarchical clustering, time of occurrence and principal component analyses. Three chromosome copy number changes were significantly more frequent in breast carcinomas from Portuguese patients, namely 17q (p=0.0016) and 20q (p=0.0164) gains and 11q (p=0.043) loss. Hierarchical cluster, time of occurrence and principal component analyses of genomic imbalances also revealed differences between the breast carcinomas of the two populations, indicating partially differing pathogenetic pathways.
We conclude that there is significant geographic heterogeneity between Portuguese and Danish patients regarding breast carcinoma-associated chromosome copy number changes.

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Bilbao, Spain, September 6-9, 2008

Genetic changes in solid tumours

Molecular cytogenetic characterization of an atypical Rhabdoid tumour with a translocation t(1;22)(p36;q11.2) involving rearrangement of *EWS1* and a deletion of *SNF5/INI1*

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Abstract

Poster presentation

Malignant rhabdoid tumors are highly malignant pediatric tumors, initially described in kidney then reported in brain and soft tissue. We describe a pediatric case of subcutaneous atypical rhabdoid tumor occurring in a 2 weeks old boy. Cytogenetic analysis disclosed two translocations t(1;22)(p36;q22.1) and t(18;22), as well as a deletion del(8p). In addition, a single nucleotide polymorphism (SNP) array revealed a loss of heterozygosity (LOH) at 1p36, 8p21.2, 8p23, 8p23 and homozygous deletion of 22q11.2 resulting in a loss of *SNF5/INI1*, a tumor-suppressor gene involved in the genesis of rhabdoid tumor. Combined results of histology, cytogenetics and genotyping led us to the diagnosis of a malignant rhabdoid atypical tumor associated with a homozygous deletion of *INI1* along with translocations involving the two chromosomes 22. 3 cases of extra renal and extra cerebral malignant rhabdoid tumors characterized by t(1;22)(p36;q11.2) with concurrent del(22q11.2) has yet been reported. One of the three cases reported in literature had a better prognosis than the more frequent poor prognosis associated with malignant rhabdoid phenotype. To date, there is no available molecular information about the breakpoints involved in this translocation. FISH characterisation using a *EWS1* specific break-apart probe revealed a rearrangement of this locus. This is the first time that *EWS1*, classically rearranged in Ewing sarcoma, is shown to be involved in this translocation.

The present study illustrates the importance of the complementarity of all the techniques available especially when the histological diagnosis is challenging. Hence, we have characterized a tumoral entity with an atypical rhabdoid phenotype, a genotype associating a deletion of *SNF5/INI1* with a translocation t(1;22)(p36;q22.1) involving *EWS1*. This specific rearrangement might explain the Ewing-like phenotype reported in certain atypical rhabdoid tumors. Further studies will be necessary to verify this hypothesis and to identify the genes involved on chromosome 1.

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Genetic changes in solid tumours

Novel variant translocation t(12;13;16) and *FUS-DDIT3* fusion in infrequent childhood myxoid liposarcoma

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Abstract

Poster presentation

The translocation t(12;16)(q13;p11) and its associated fusion transcript *FUS-DDIT3*, characterize more than 95% of cases myxoid liposarcoma . This cytogenetic finding is used to differentiate myxoid liposarcoma from other myxoid tumors when pathologic findings are confusing or indecisive. Other chromosomes could be involved in rare variant forms of the translocation (little data are available).

We report a 15 years old patient with pathologic evidences of a myxoid liposarcoma. Since this tumor is principally an adult tumor, cytogenetics approach was used to confirm the myxoid liposarcoma diagnosis. GTG banding karyotype showed the structural rearrangement of chromosomes 12, 13, and 16 suggesting a complex translocation t(12;13;16). Chromosome painting for chromosomes 12, 13 and 16 confirmed the variant translocation. Furthermore, using BACs clones covering *FUS* and *DDTI3* genes, FISH experiment demonstrate both genes rearrangements and revealed the fusion *FUS-DDIT3*. These results illustrate that in addition to pathological findings, conventional cytogenetics and FISH provide more complete characterization of an myxoid liposarcoma in a young patients.

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Genetic changes in solid tumours

Characterization of primary melanomas using high-resolution array based CGH technology

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Abstract

Poster presentation

Malignant melanoma is an aggressive heterogeneous disease for which new biomarkers for diagnosis and clinical outcome are needed. We investigated by array-CGH the presence of DNA gains and losses to provide better genomic profile of primary malignant melanoma and to explore the possibility to distinguish metastatic from non metastatic melanomas using this technology.

Experimental Design: High resolution array-CGH (Agilent Technologies, Palo Alto, CA), with more than 40.000 probes, has been used to analyze 20 frozen tissues of vertical growth phase primary melanoma with a minimum follow-up time of 36 months. Eight patients developed nodal metastatic disease and twelve did not. For results validation, 83 additional melanoma samples with similar clinical characteristics were analysed by FISH.

Results: DNA copy number aberrations (CNA) were observed in 19 out of 20 cases. The most frequent changes were complete or partial losses in chromosomes 9 (12 cases, 60%) and 10 (9 cases, 45%), partial gains or trisomies of chromosome 7 (10 cases, 50%); and monosomy of chromosome 19 (7 cases, 35%). Sixty-four recurrent aberrant regions (SORIs) were precisely delimited and used as variables for clustering. Unsupervised Cluster analysis allowed the segregation of samples into two genomic groups that naturally fitted with the metastatic condition of the cases. Four of these aberrant regions were chosen for their biological interest and were confirmed as aberrant using FISH technique on fixed paraffin embedded tissues.

Conclusions: Supervised classification allowed obtaining aberrations useful to separate samples with different clinical outcome. Obtained results are useful to improve the knowledge about melanoma tumorigenesis but unfortunately cannot be used as a marker for metastatic progression.

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Genetic changes in solid tumours

Identification of novel genes involved in colorectal cancer predisposition

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Abstract

Poster presentation

Colorectal cancer (CRC) is the second most common cancer in the Western world in terms of both incidence and mortality rate. A positive family history of CRC is observed in about 20 to 25% of the cases. High-penetrant germline mutations in APC, MUTYH and the mismatch repair genes MLH1, PMS2, MSH2 and MSH6 account for less than 5% of hereditary cases whereas in the majority of these families the genetic defect is still unknown. In order to identify novel high-risk mutations contributing to CRC predisposition we employed genome-wide copy number profiling using high-resolution SNP-based arrayCGH on normal tissue DNA from 32 independent patients with microsatellite-stable CRC without polyposis. All patients were suspected for hereditary CRC because of their young age at diagnosis or their positive family history for CRC. We identified small (100-160kb) copy number anomalies in five independent families (16%), in all the cases affecting only a single gene. None of the genes had previously been described to be involved in colorectal cancer susceptibility. All genomic lesions were validated with multiplex ligation-dependent probe amplification (MLPA). In four cases we were able to establish that the aberrations were inherited from one of the parents. Two of the genomic lesions were deletions affecting a microRNA gene, illustrating that constitutional defects in these gene expression regulators might be common. Interestingly, at least two of the identified genes could be linked to pathways involved in CRC development. By screening a second, much larger cohort of independent families with suspected familial CRC (n=246) we thus far found at least one of the genes to be recurrently affected, which strongly supports its role in CRC predisposition.

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Genetic changes in solid tumours

Value of combined array CGH and cytogenetic analysis for a precise characterization of childhood and adolescent embryonal tumors and sarcomas

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Abstract

Poster presentation

Background: The value of conventional cytogenetics for the diagnosis and prognosis of solid tumors has been demonstrated. Microarray Comparative Genome Hybridisation (array CGH) allows for the detection of copy number changes throughout the entire genome at a resolution that far exceeds that of cytogenetics. Array CGH studies, especially of pediatric solid tumors, are still scarce.
Methods: We analysed 60 patients from a single center with embryonal tumors and sarcomas using conventional cytogenetics, array CGH and M-FISH. We have correlated our findings with standard morphological histopathological analysis, clinical features and outcome.
Results: With this combined approach we have detected non random aberrations involving 1q and 2q, +8, +20, a t(1;6)(q21;q26) and a der(4)t(1;4)(q25;q34) in hepatoblastomas; 1q gain, del(1p), monosomy 22, aberrations involving 16(q13-qter), i(7)(q10) and a previously not published t(2;8)(p1.5;q22.3) in nefroblastomas (Wilms) tumors; a del(1p), the typical t(11;22)(q24;q12), secondary aberrations der(16)t(1;16), +8, +12 along with an atypical t(4;19)(q32;q13.2) in Ewing sarcomas/PNET and a complex karyotype t(2;5),t(12;20),t(X;18;21) along with the typical t(X;18)(p11.2;q11.2) and a previously not published t(11;22)(q24;q12) in synovialosarcomas.
Conclusions: Our findings bring further evidence regarding typical hepatoblastoma abnormalities, so far scarcely reported, with array CGH analysis refining the resolution of conventional cytogenetics. The detected changes in the series of nefroblastoma patients support the already existing evidence concerning prognostic significance of chromosomal aberrations in this tumor. The impact of secondary aberrations on prognosis in Ewing sarcomas is still a matter of debate. Altogether, the value of the precise characterisation of pediatric tumor biology lies in the future possibility to assist the development of more specific targeted drugs. Supported by grant IGA MZ c.NR/9050-3

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Genetic changes in solid tumours

Chromosomal instability in osteosarcoma

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Abstract

Poster presentation

Osteosarcoma (OS) is the most common primary bone malignancy, and is characterized by chaotic patterns of chromosomal aberration with considerable cytogenetic heterogeneity at the cellular level. By integrating molecular cytogenetics with profiling approaches [comparative genomic hybridization (CGH), spectral karyotyping (SKY), or multicolor banding (mBAND)], together with classical G-banded cytogenetics analysis of OS tumors, we and others have been able to better describe consistent features and complexities of the OS karyotype and genome. Collectively, these studies have shown that all chromosomes could be a target of chromosomal instability (CIN), but some genomic regions are more prone for rearrangements and copy number change than others, namely: 1p35-p36; 6p12-p21; 8q23-q24; 17p11-p12 and 19p13. This unstable genome is considered to provide an adaptive advantage to OS, so that tumors are able to rapidly acquire resistance to chemotherapy, and similarly clonal selection will favour OS cells with a propensity to metastasize. Many mechanisms have been proposed to explain the generation of chromosomal diversity, including deregulation of DNA maintenance, repair deficiencies, defects of check points, centrosomes, spindle attachment, cytokinesis, and/or other segregation anomalies. We have developed a strategy to rank OS tumors utilizing both their level and degree of complexity that contributes to CIN. The approach combines relative indices of genomic imbalance detected using high resolution array-CGH, complemented by cellular measures of CIN determined by interphase Fluorescence In-Situ Hybridization (FISH). Representative analyses of chromosome 8 using a series of 10 OS tumors classified and ranked for the CIN phenotype based on a combination of numerical/ structural chromosomal changes and incorporating the extent of cellular heterogeneity. It is proposed that clustering of tumors based on relative measures of CIN will be an important step in delineating the critical pathways leading to OS oncogenesis. This model can be extended to include other types of genome-wide approached such as epigenetic, gene expression, and miRNA profiling to provide a comprehensive description of the cause and consequences of chromosomal instability in OS.

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Genetic changes in solid tumours

Biologically relevant copy number alterations in osteosarcoma cell lines: An array comparative genomic hybridization analysis

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Abstract

Poster presentation

Although cytogenetic analyses in osteosarcoma (OS) revealed complex chromosomal rearrangements with a markedly cell-to-cell variation, no specific recurrent numerical or structural chromosomal rearrangement has been identified so far. We employed cDNA microarray containing 16,000 cDNA clones to perform a genomewide high-resolution analysis of seven human OS-derived cell lines to identify gene copy number patterns that could be of importance in the tumorigenesis of this neoplasm by comparing our results with to those previously found in 21 primary OSs in our laboratory. Changes were considered reliable only when five consecutive clones were altered in 20% of cases. Complex patterns of changes of DNA copy number sequences were observed in all cell lines (22.6±3.6 amplified/deleted chromosomal locations/sample). Amplicons were almost as frequent as deletions (amplicons: deletions = 1.0:0.9), with a mean value of 12.0±2.8 of aberrations/sample. Recurrent amplified genes seen in both cell lines and primary tumors mapped to 1p22.p31, 1q21.q22, 2q11.2.q13, 8q21.1.qter, and 17p11.2.p12 whereas 6q16.q21 was found commonly affected by losses. Further molecular studies focusing the analyses to those recurrent amplified and deleted chromosomal locations observed in both cells lines and primary OS will most probably reveal those important target genes involved in the tumorigenesis of OS.

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Genetic changes in solid tumours

Wide-genome analysis identified gain of 1q as potential negative prognostic marker for survival in high-risk disseminated neuroblastoma

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Abstract

Poster presentation

Neuroblastoma (NB) is the most common, extra-cranial pediatric malignant solid tumor and it is responsible for about 15% of childhood cancer-related deaths. NB is characterized by a remarkable heterogeneous clinical behavior, ranging from aggressive metastatic disease (stage 4), accounting for 50% of all NB patients, to spontaneous regression, which mainly occurs in infants. Patients with localized disease have good overall survival (95% for stages 1 and 2, 65% for stage 3) and progression of disease in stage 4 infants is generally halted by good response to therapy. Conversely, despite multimodal highly intensive chemotherapy, relapse occurs in more than half of patients older than 1 year of age at diagnosis with stage 4 NB, with a 3 year survival rate of 25%. In order to identify novel molecular prognostic markers useful to refining current criteria of patients´ relapse risk estimation, we performed a wide-genome analysis of stage 4 NB tumors of patients over 1 year of age at diagnosis and with at least 3-years of follow-up, who i) died with disease (Group "DWD", n=27) or ii) survived (Group "S", n=15). NB samples were investigated at diagnosis using array-comparative genomic hybridization (44k microarrays, Agilent Technologies). Tumors of group S showed 61 numerical aberrations (average 4/case, range 1-16) and 32 structural aberrations (average 2.1/case, range 011). The most frequent abnormalities were 17q gain (9/15) and 7 gain (6/15). All tumors of group DWD had at least one structural aberration (average 7.7/case, range 1-18). The most frequent changes were 17q gain (21/27), 2p gain (14/27), 11q loss (13/27), 1p loss (12/27), 1q gain (10/27), and 3p loss (9/27). In contrast to group S, numerical aberrations were present with a lower frequency in tumors of group DWD (average 2.9/case, range 0-8), mainly represented by gains of whole 7 (12/27), 18 (9/27), and 19 (6/27) chromosomes. Hence, although all tumors were characterized by complex aberration patterns focused on chromosomes 1, 2, 3, 7, 11, and 17, the proportion of structural copy number alterations was higher in NBs of group DWD compared to group S. Since no reliable marker is presently able to predict outcome of patients older than 1 year of age suffering from metastatic NB, we defined correlations among molecular profiles of tumors and clinical features of patients. In our cohort the strongest indicator for outcome was gain of 1q (minimal common region of gain: 1q24.3-q42.2). Such structural aberration resulted significantly predictive of OS (P = 0.0012) and EFS (P = 0.0016), suggesting that genes on 1q are related to poor survival in high-risk NB.

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Genetic changes in solid tumours

Chromosomal imbalances identified by CGH in invasive versus non-invasive bladder tumours

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Abstract

Poster presentation

Bladder cancer is the fifth most frequent neoplasm in developed countries. The most common form is bladder urothelial cell carcinomas (BUC), accounting for approximately 90% of the cases. BUC are usually classified in two categories: around 70-80% are non muscle invasive (stage Ta and T1) at initial diagnosis and they are associated with high risk of recurrence (70%) following treatment. The remaining 20-30% of bladder cancers show muscle invasion at the time of diagnosis (≥ T2).
The objective of the present study was to describe the chromosomal changes associated to invasiveness in BUC. Comparative Genomic Hybridisation (CGH) was performed on 187 bladder urothelial cell carcinomas (BUC); 82 Ta-T1 low grade, 30 Ta high grade, 25 T1 high grade, 34 T2-T4 no anaplasic and 16 T2-T4 anaplasic. Statistical analysis was performed using the Fisher exact test. Overall survival was estimated with the Kaplan-Meier method. The survival curves were statistically compared by a log-rang test. Tumours were organised in two groups: non muscle invasive (137/187) and muscle invasive (50/187). Five of the chromosomal alterations observed in both groups showed statistically significant differences ($p \leq 0.05$) when compared. Loss of 9q was found at a frequency of 27.74% in non muscle invasive tumours, in front of the 8% in muscle invasive tumours, confirming that this lost is an early event in bladder carcinogenesis. The four remaining chromosomal alterations, -4p, +6p, +10p and +14, were more frequently found in muscle invasive tumours. This suggests that these chromosome regions could contain genes involved in invasion processes. Interestingly, Kaplan-Meier analysis showed that gain of 6p was associated with overall poor survival ($p = 0.016$).

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Genetic changes in solid tumours

Is there constitutional chromosome instability in patients affected by two or more primary tumors and/or a family history of cancer?

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Abstract

Poster presentation

Chromosome instability in peripheral blood lymphocytes (PBLs) may be associated with cancer predisposition. Such chromosome instability can be detected by a high rate of spontaneous alterations or an increased sensitivity to mutagenic or clastogenic agents.

To investigate this hypothesis, we selected 24 bladder cancer patients with two or more primary tumours and/or a family history of cancer and 14 controls matched to the cases regarding sex, age and smoking status. We analysed the expression of fragile sites (FS) induced by aphidicolin and specific aberrations of the *CCND1* gene and the 9p21 locus by FISH. The frequency of fragile site expression in PBLs of the patients was higher than in controls, but differences were not significant (3.39 FS/cell versus 2.97, respectively). Interestingly, a subgroup of patients showed a frequency of FS expression per cell higher than 5. Significant differences were observed for the expression of fragile sites between smokers patients and smokers controls [95% confidence interval (95% CI) 1.212 to 2.581] and an odds ratio (OR) of 1.768. These results suggest that the patients present major susceptibility of the putative effects of tobacco in FS expression. Gain of *CCND1* was found in 58.3% of the patients and in 30.8% of the controls. Loss of 9p21 was detected in 12.5% of the patients and 7.7% of the controls. We only found significant differences for *CCND1* and 9p21 alterations when comparing smokers versus non smokers, both in patients and in controls. These results not only support the susceptibility of the 9p21 locus to mutagenic effects of tobacco but also suggest a putative effect on the *CCND1* gene.

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Genetic changes in solid tumours

Genetic characterization of progression in embryonal rhabdomyosarcoma. Comparative genetic analysis of primary and recurrent or metastatic tumors

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Abstract

Poster presentation

Rhabdomyosarcoma (RMS) is one of the most common solid tumours in children. Embryonal and alveolar subtypes of RMS present completely different genetic abnormalities. Cytogenetically, embryonal RMS (eRMS)is most frequently hyperdiploid, with extra copies of chromosomes 2, 7, 8, 11, 12, 13, and 20, and is characterized by loss of heterozygosity on the short arm of chromosome 11 (11p15.5), suggesting inactivation of a tumour-suppressor gene. No consistent structural chromosomal alteration has been identified. In contrast, the majority (80-85%) of the alveolar RMS (aRMS) have the reciprocal translocations t(2;13)(q35;q14) or t(1;13)(p36;q14). t(2;13) appears in approximately 70% of patients with the alveolar subtype. The molecular counterpart of this translocation consists of the generation of a chimeric fusion gene involving the PAX3 gene, located in chromosome 2, and a member of the fork-head family, FOXO1 (formerly /FKHR/), located in chromosome 13. A less frequent variant translocation t(1;13) involves another PAX family gene, PAX7, located in chromosome 1, and FOXO. Furthermore, a number of oncogenes, primarily MYCN and MDM2 have been found amplified in RMS. Although embryonal rhabdomyosarcoma represents the most frequent form of RMS, the karyotypic characterization of this tumor subtype is still incomplete. We report the karyotypic analysis of three new cases of infant-onset eRMS and include the cytogenetic and molecular changes observed in recurrences and/or metastasis. The patients were three children (age 8 months to 4 years). The tumors recurred between 15 and 20 months after diagnosis, and one of them metastatized to lymph nodes. Samples of primary tumors, recurrences and metastasis were studied by histology, immunohistochemistry and cytogenetics. A fragment of each sample was cryoconserved for molecular studies. Two cases had a hypotriploid karyotype and one was hyperdiploid. DNA content was also analyzed by flow cytometry showing DNA indexes concordant with the cytogenetic analyses. All cases showed in common gain of chromosomes 8 and 11 and the presence of structural abnormalities, all of these unbalanced rearrangements. One case showed genomic amplification as dmin. Subsequent molecular analyses showed tumor cells to be negative for FOXO1A, PAX3, or PAX7 gene locus rearrangements and also negative for PAX3-FOXO1A and PAX7-FOXO1A fusion transcripts by RT-PCR Analysis of the regulatory genes responsible for G1-to S-phase transition showed a homozygous deletion of the INK4a and INK4b locus genes in a spindle-cell eRMS. Amplification of Cyclin D1 and MDM2 was also observed.

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Genetic changes in solid tumours

Genetic and biological characterization of a novel human melanoma cell line (*MEL-RC08*)

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Abstract

Poster presentation

Recent investigations have revealed distinct patterns of genetic changes in different groups of cutaneous melanomas, as defined by anatomical site and pattern of sun exposure. A novel human malignant melanoma cell line, designated mel-rc08, was established from a cranial metastasis of a malignant melanoma of the skin. Tumor tissue sample was disaggregated using 0.02% collagenase type II The cells were seeded into culture flasks containing 5 ml of complete medium (RPMI-1640, supplemented with 10% FBS, 1% L-glutamine, penicillin, and streptomycin sulfate. A confluent cell layer was obtained at day 7 of culture. Subcultures were then made with 0.25% trypsin-EDTA Cells of all passages were stored under liquid N_2 in culture medium containing 5 % DMSO. Normal growth kinetics was determined by tripsinizing the cultures in triplicate and resuspending the cells in medium. The viable cells were counted every 24 hours for 4 days. Doubling time of the cell population was estimated in a logarithmic growth phase. The cell line was analyzed by histology and cytogenetics methods, flow cytometry and FISH analysis. The first passage of the cell line took 7 days. The cells were passaged routinely in vitro for more than 145 generations. They had a slightly elongated shape and grew in monolayer. The cells showed a saturation density of 8.8×10^4 and a plating efficiency of 95%. The mitotic index was 10 mitoses in 10 LPF. Finally, after single cell cloning, five cell lines showing similar phenotypes were analyzed. The cells are aneuploid, and the modal chromosomal number is in the hypertriploid range with polysomies of chromosomes 1, 7, 8, 18, 20, 21, 22, and X. Clonal structural abnormalities affecting chromosomes: 1, 2, 3, 4, 5, 6, 8, 9, 11, 13, 14, 15, 17, 21 and 22 were observed. These rearrangements have been analyzed by conventional cytogenetics and spectral karyotype (SKY). DNA flow cytometry has demonstrated an aneuploid peak with a value of 1.51 DI corresponding to a triploid range. Molecular cytogenetics using dual-color mixture consisting of p53 (17p13)/SE17 and p16 (9p21)/SE9 (KREATECH) showed no deletion of these genes. In conclusion genetic instability of melanoma was shown by the high percentage of polysomies, the variation of the ploidy status and the high number of rearrangements found. The *mel-rc08* cell line is well characterized and could be a very useful material for basic research of malignant melanoma. Supported by: FIS07/1203, FISPI070805 and SAF 2005-04340 (MEC)

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Genetic changes in solid tumours

Molecular cytogenetic of hepatic metastasis in colon cancer patients: correlations with the prometastatic and proangiogenic potential Diagnostic-therapeutic implications

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Abstract

Poster presentation

Introduction: Colon cancer is the third most common type of cancer in the world. Several reasons contribute to its high mortality including the difficulty in determining if patients will develop local recurrences or distant metastases at the time of diagnosis and knowing whether intermediate staged primary carcinoma patients with no clinical or histopathological signs of metastases should receive chemotherapy or radiotherapy after surgical resection. Despite solid references reporting chromosomal alterations for this type of cancer, the prediction of the illness remains unclear and therefore the search for more reliable novel DNA biomarkers is needed. AIM: The main goal of the present study is to identify novel prognostic and diagnostic biomarkers which are capable of making a colorectal cancer diagnosis and predicting the liver metastases commonly associated with it.
Methods: The search for novel gene alterations was carried out using liver metastasis from 10 patients by means of Comparative Genomic Hybridization arrays (CGHa). The technology used was Agilent Human Genome CGH Microarray that includes 40000 providing genome-wide coverage with an average spatial resolution of approximately 75 kb, including coding and non-coding sequences. The pre-selected markers were further evaluated in 50 different frozen colorectal carcinoma hepatic metastases by quantitative PCR and the results were analyzed by an absolute method adapted by Moody et al. (2000), capable of determining the gene copy number in each sample. The validation of the cytogenetic marker candidates was performed in a broad range of hepatic metastasis samples embedded within a tissue micro array by Fluorescent In Situ Hybridization (FISH) using self-made DNA probes labelled with Alexa Fluor 488 fluorophore by nick translation methodology. Nuclei were counterstained with DAPI and samples were analysed using an Olympus BX61 fluorescent microscope.
Results: Following the described methodology, we were able to identify set of ten novel genes with copy number alterations. Six of them were found amplified in the tumour cells from hepatic metastases of patients with colon adenocarcinoma in at least 30% of the cases studied. Another alteration was present in 92% of the cases studied. Another four markers presented deletions, representing one of these up to 43% of the analyzed patients. All the amplifications found on this study are associated with proliferation, growth and cell signalling cellular processes, whereas the loss of heterocigosity (LOH) alterations found are involved in cell cycle control regulations. The number of copies for a particular gene was optimized by using the quantitative PCR method. Using tissue micro array technology we are currently validating these selected genes in larger populations by means of FISH technique.
Conclusion: We have been able to corroborate the numerical alterations found by CGHa methodology and the following validation of pre-selected markers was been carried out using standardised Q-PCR and FISH protocols

and tissue microarray technology. The prognostic value of the multi-probe set of genes will be assessed to design a more predictive multi-FISH approach related to clinical outcome in colorectal cancer.

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Genetic changes in solid tumours

Significance of molecular cytogenetic characterization of glioblastoma for prediction of patient prognosis

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Abstract

Poster presentation

Introduction: In our work we have proved the significance of cytogenetic and molecular genetics for glioblastoma diagnostics, treatment modifications, and prediction of patients' prognosis.
Material and Methods: In the years 2001-2006 we carried out molecular cytogenetic examinations of biopsy samples of 32 patients with GBM The results were correlated with patient survival.
Results: We investigated chromosomal abnormalities in touch preparations of the tumours by interphase-fluorescence in situ hybridization (I-FISH). The results showed monosomy 10 in 100% of cases, polysomy 7 (3-5copy of chromosome 7) in 93% of cases, EGFR gene amplification was present in 26% of cases and p53 gene deletion in 22% of cases. Chromosomal abnormalities of 22 cases of GBM were analyse using CGH. A total of 99 different changes were detected (with a median of 5 changes per case). The most prominent gains were found in chomosomes 7, 19, 3q, 12, Xp. The losses affected especially the chromosomes 10, 6, 13q, 14q, 1p. Losses of the chromosomal material (total of 56) were more frequent than gains (total of 43). A total of 172 abnormalities were found by HR-CGH (with a median of 9 changes per case). Among the most frequent deletions areas was deletion 10q (78%), 10p (64%), 9p21 (44%), 14q21-q22. Amplifications were frequent in sections 7p (69%), 7q (64%) a 19q. We proved two locations with significant amplification 7p12 (gene EGFR) and 12q14 (geneM2D2). The shortest survival time was observed in patients where shared chromosome 7 polysomy was observed with monosomy of chromosome 10 and EGFR gene amplification.
Conclusions: Glioblastoma is a polygenic disease, it is caused by a great number of genes on different chromosomes.The effects add up and therefore it is more complicated to intervene therapeutically.

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SESSION

Mechanism underlining tumourigenic genetic changes

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11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours

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Mechanism underlining tumourigenic genetic changes

Comparative cytogenetic analysis of mouse models for breast cancer pinpoints to the amplification of the novel oncogene Septin 9

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Abstract

Oral presentation

In order to identify oncogenes and tumor suppressors important for breast tumorigenesis and maintained trough evolution we performed comparative cytogenetic analysis on more than 90 primary adenocarcinomas from six murine breast cancer models transgenic for the oncogenes *c-Myc* or *Her2/Neu*, knock-out for the tumor suppressor gene *Brca1* or expressing viral proteins such as polyoma virus middle T antigen or SV40 large T antigen under control of the mammary gland tissue specific promoter MMTV (MMTV-PyMT, SV40C3(1)/Tag, BRCA1$^{ko/co}$, MMTV-*cmyc*, MMTV-*Notch4* and *HER2/neu*). Our comparative cytogenetic analysis revealed that in general, the pattern of genomic imbalances in experimentally induced mouse mammary gland adenocarcinomas is similar to human breast cancer. In particular we detected high copy number gains of the Septin 9 (*Sept9*) locus that occurred in the form of double minute chromosomes, duplications and jumping translocations all resulting in *Sept9* over-expression. Septins are a family of cytoskeleton related proteins that form a network with actin, tubulin and intermediate filaments. *Sept9* is one of 14 members of the septin gene family that were first identified in budding yeast where they form a highly specialized cortical domain that binds to microtubules and separates the mother cell and the bud during cytokinesis. *Sept9* function in normal and cancer cells remains largely unknown. In the effort of validating our mouse models findings in human breast carcinogenesis we now show that *Sept9* function as a potential biomarker for tumorigenesis, and that differential expression of the *Sept9* gene is present in polarized epithelial cells of normal breast when compared to DICS (ductal in situ carcinoma). Additionally using a novel bioinformatic tool to detect high-density CG di-nucleotides regions in the genome, we identified CG clusters mapping to isoform transcription start sites thereby prompting the hypothesis that methylation at specific CG di-nucleotides is one of the mechanisms involved in the regulation of isoform expression. A synergistic approach combining proteomic, genomic and epigenetic analyses was implemented to decipher the mechanism regulating the expression of the multiple *Sept9* isoforms. These experiments will generate insights into the epigenetic regulation of *Sept9* isoform expression and its potential role in cancer development.

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Mechanism underlining tumourigenic genetic changes

Molecular characterization of pediatric medulloblastoma by combining genomic and gene expression profiling

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Abstract

Oral presentation

Purpose: Medulloblastoma (MB) shows both numerical and structural chromosome abnormalities as well as abnormal gene expression. Combining data from genomic and gene expression microarray analyses could allow to identify genes whose expression is altered by chromosomal gains or losses. Such overlay analysis is useful to identify candidate genes associated to MB pathogenesis.
Methods: We performed genome-wide analysis of 24 classical MBs by Agilent 244K oligonucleotide microarrays. Images were processed by Feature Extraction v.9.5 and data files were visualized by CGHAnalytics v.3.4 software. In 14 out of 24 samples we used Agilent 44K microarrays to establish gene expression profiles. Quality control and data normalization were performed using GeneSpring GX v9.0 software. Tumor expression data were compared to a commercial normal cerebellum RNA pool. Genes whose expressions in at least 13 tumor samples were 3-fold greater or smaller than expression level in normal cerebellum were selected according to functional annotation analysis.
Results: The most frequent gains occurred at chromosomes 7q (78%), 17q (50%), 1q (47%), whereas 8p21.2-pter and 17p11.2-pter regions were lost in 39% and 20% of cases respectively. Four MBs showed gain of chromosome 2 and 3 cases showed amplification restricted at MYCN locus. Gene expression analysis identified 540 and 737 genes that were up-and down-regulated, respectively.
Conclusion: Genome-wide analysis showed complex DNA imbalances with high frequency of 17q and 1q gain. High TOP2A, BIRC5 and CDC6 expression was found associated with 17q gain. Our data indicate that expression of MB-associated gene signature is consistent altered by copy number changes. Gene Ontology analysis indicated a higher cellular proliferation activity in MB cells respect to normal cerebellum. Conversely, down-regulated genes belong to nervous system development, cell communication and neuron differentiation pathways, indicating a deregulation of cell differentiation in MB pathogenesis.

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Mechanism underlining tumourigenic genetic changes

Chromosome instability in bladder cancer: Centrosome abnormalities and CCND1 gene amplification

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Abstract

Oral presentation

Amplification of chromosome band 11q13 is commonly observed in a number of human cancers, including bladder cancer. Despite a large number of reports regarding abnormalities of this chromosome band, very little has been done to clarify the behaviour and the fate of the amplicon during tumor development.
In the present study, Fluorescence *In Situ* Hybridization (FISH) with *CCND1* probe and immunolabelling of α, β and γ tubulin on 22 paraffin-embedded bladder tumors were performed. The careful observation of metaphasic cells allowed us not only the detection of 11q13 amplification, but also the discrimination between different types of homogeneously staining regions (hsr). This is the first report in the literature showing that *CCND1* gene amplification can occur on double minutes (dmin). The simultaneous presence of dmin and hsr inside the tumor, suggests that dmin originates from pre-existing hsr which becomes unstable, giving rise to hsr fragments which, in turn, derive to dmins, which are spontaneously eliminated by micronuclei extrusion. Chromosome instability correlated with centrosome abnormalities ($p<0.05$). Several cytogenetic alterations such as chromosome laggard, anaphasic bridges and dicentric chromosomes were also observed. Only cases with *CCND1* amplification on dmins showed a short survival. The fact that the non-muscle invasive tumors which actively extrude the dmins by micronuclei are those from the patients with the worst outcome, stresses the importance of further investigation of *CCND1* amplification form in bladder cancer.

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Mechanism underlining tumourigenic genetic changes

Functional validation of genomic and transcriptome profiles identifies candidate oncogenes in colon cancer

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Abstract

Oral presentation

Copy number changes are very common in most solid tumors. Previous conventional CGH studies invariably showed that the gain of chromosome 13 is a major aneuploidy in colorectal tumorigenesis. However, less is known about the genes that might lead to the recurrent selection for gain and/or amplification of this chromosome. The analysis of gene expression along with an RNA interference screening allowed us to describe potential novel oncogenes on chromosome 13. In order to detect minimal regions of amplification, we performed high-resolution microarray CGH of 31 colon cancers. For this purpose, we used a microarray with 185K oligonucleotides that covered the human genome (Agilent Technologies). Minimal regions of recurrent amplification were detected at 13q12.13-q12.3 and 13q34. A whole genome 44K microarray (Agilent Technologies) was used to assess the gene expression in a subset of 25 primary colon cancers and 15 colorectal cancer cell lines in order to identify overexpressed genes that map to the two regions of amplification. A total of 44 genes located at 13q12.13-q12.3 and 13q34 were detected to be overexpressed compared to normal colon. These results were validated by RT-PCR in 25 colorectal cancer cell lines. Then, a primary RNAi screening for the 44 candidate genes was performed in triplicate using two different siRNAs per each gene. Functional validation assessing cell viability after 72 and 96 hours was performed in the aneuploid colon cancer cell lines SW480 and HT29, and in DLD-1, which is diploid. Cell viability was quantified using CellTiter-Blue. Remaining RNA after silencing was assessed by RT-PCR Out of the 44 genes, RNA silencing of 10 genes showed a reduction of at least 20% of viability (up to 70%) in at least two cell lines for at least one siRNA A secondary screen was performed using two additional siRNAs for those 10 genes in order to confirm the results of the primary screen. Among these 10 genes, two have an unknown molecular function in the GO database. On the other hand, we identified two transcription factors, an rRNA processing protein, two receptor activity proteins, a nuclear pore complex protein, an amino acid transporter, and a protein/DNA binding gene product. Individual gene silencing for each gene using two different siRNAs, followed by gene expression profiling for each knockdown product and pathway analysis, will allow us to identify the involved cellular processes and their potential relevance in colorectal tumorigenesis.

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Mechanism underlining tumourigenic genetic changes

Multidisciplinary evaluation of the role of reciprocal translocation and gene fusion in the pathogenesis of solid tumours

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Abstract

Oral presentation

Based in the available genetic and cytogenetic literature, it is widely assumed the hypothesis of the chromosome translocations (CT) and their effect on a specific oncogene expression as being a main player in the development and progression of all types of cancer. However, our knowledge about CT in solid tumours is clearly less relevant than in leukaemia and sarcomas.

Purpose: Using literature information and in-silico analysis we have elaborated a list of gene that could be susceptible to be rearranged in pancreas and bladder cancer.

Experimental Design:

(1) From the literature, we have selected a list of 6 genes with a high probability to be involved in RT. We designed break-apart FISH probes and we have tested the status of theses genes in tissue microarray (TMA) contain 112 samples of pancreatic carcinoma.

(2) For the in-silico approach, we have adapted a bioinformatics system to find genes that could be involved in RT, analyzing gene expression array data of bladder and colon cancer. Cancer Outlier Profile Analysis (COPA) algorithm was used to assign the probability to each found gene to be a partner in the translocation events.

Preliminary results:

(1) MLL, ETV6, EWRS1, ERG, ERS, ICAM were selected from the literature and tested in the pancreatic tumour TMA Eleven per cent of the tumours showed an irregular pattern of signals with the ETV6 probe. These samples are being currently re-analyzed to confirm the rearrangement and to try to disclose the aberration. MLL and EWRS1 have not shown any positive result. The remaining genes are under study.

(2) 36 genes were identified by the in silico approach for the bladder cancer study. Break-apart FISH probes are being designed to test in TMA

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Mechanism underlining tumourigenic genetic changes

The renal cell carcinoma-associated oncogenic fusion protein PRCCTFE3 induces $p21^{WAF1/CIP1}$ mediated cell cycle arrest

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Abstract

Oral presentation

As a result of the renal cell carcinoma-associated t(X;1)(p11;q21) translocation the transcription factor *TFE3* gene on the X chromosome is fused to *PRCC* gene on chromosome 1. Previously, we found that the PRCCTFE3 fusion protein, which has retained all known functional domains of TFE3, acts as a more potent transcriptional activator than wild type TFE3 and that PRCCTFE3 expression leads to *in vitro* and *in vivo* transformation of certain cell types, including those of the kidney. From these results we concluded that PRCCTFE3 acts as an oncogenic protein. Here we show that the primary response of exogenous PRCCTFE3 expression is cell cycle arrest. This arrest appears to be mediated by induction of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$. In addition, we found that this arrest is p53 independent. This observation is in line with several published reports indicating that the initial response of cells to an oncogenic event is the acquisition of a senescent phenotype through induction of cell cycle arrest. Subsequently, this senescent phenotype may be numbed, thus leading to full-blown tumor formation.

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Mechanism underlining tumourigenic genetic changes

Alternative splicing of *ERG* and *TMPRSS2:ERG* rearrangement in prostate cancer cell lines

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Abstract

Poster presentation

Fusion of the *TMPRSS2* gene with the ETS transcription factor *ERG* is present in more than 50% of the prostate carcinomas. Despite of the diversity of the alternative splice transcripts described for *ERG*, the pattern of *ERG* transcripts expressed by prostate cancer cells has not yet been determined.

We have evaluated the expression pattern of *ERG* and *TMPRSS2:ERG* chimeric transcripts in different prostate cancer cell lines, positive (VCaP) and negative (LNCaP, DU145 and 22Rv1) for the *TMPRSS2:ERG* rearrangement using RT-PCR analysis followed by sequencing of amplification products. Analysis of exons 5 to 11 revealed, in addition to the full-length *ERG* transcript, expression of one alternative splice *ERG* transcript in all cell lines studied, resulting in deletion of exon 8 (del_ex8). A second alternative splice *ERG* transcript was expressed only in DU145 cells, with deletion of both exons 7 and 8. In VCaP cells, amplification of *TMPRSS2:ERG* transcripts revealed a complete *TMPRSS2:ERG* type III transcript and three smaller products that proved to be alternative splice variants of this chimeric transcript. Besides the del_ex8 transcript also identified in wild-type *ERG*, two new splice variants were identified, where different fractions of *ERG* exon 4 were found in-frame with different fractions of *ERG* exon 10. In both cases the “fusions” occurred within 6 bp that were common to both exons.

We concluded that the *TMPRSS2:ERG* fusion transcript shows several alternative splice variants in VCaP prostate cancer cells and that *ERG* transcripts missing exon 8 are detected in prostate cancer cells both with and without the fusion gene.

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Mechanism underlining tumourigenic genetic changes

Expression profile of significant immortalization genes in colon cancer

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Keywords: telomeres, immortalization, colon cancer, genomic instability, microarrays

Abstract

Poster presentation

Background and Aim: Cancer is described as a disease of genomic instability, a multistep process involving multiple mutations and chromosomal aberrations. Telomeres are highly specialized structures at the ends of chromosomes whose function is to stabilize and protect the ends of linear chromosomes, therefore determining cell immortalization. Homeostasis of telomere length is a multifactor-dependent process of forming and maintenance of telomere structure in which telomerase is involved along with other determinants. Since cellular immortalization is an early and obligatory step towards cancer, the role of telomere dysfunction in initiating and promoting chromosomal instability is a main point of interest in our study in the context of understanding early steps of carcinogenesis and searching early clinical markers of cancer.

The aim of our study is to determine immortalization genes that are significant of colon cancer and assess their usefulness in the early diagnosis of this tumour.

Methods: The study has been divided into two parts: part I -searching for the significant genes of colon cancer with expression profile examination of immortalization genes, part II -verification of results with quantitative assessment of the expression of suspected genes (still on-going). In part I the oligonucleotide microarrays (Affymetrix) methods were applied in 13 probes of colon cancer (low and high clinical staging, all G2 grading, all adenocarcinoma typing) and 9 probes of controls (normal colon tissue). Expression profiles of 119 transcripts known to be involved in cell immortalization process were compared between the groups with use of Significant Analysis Microarray (SAM) software for determining significant genes. A number of the immortalization genes closely specific to telomere homeostasis were chosen for quantitative verification in part II of our study by QRT-PCR in a larger population.

Results: Part I: we defined 23 genes with significantly different expression between high clinical stage colon cancer and control group, and 22 between low clinical stage colon cancer and control group. For further analysis with QRT-PCR we selected three genes showing altering expression in both low and high clinical stage colon cancer: *ACD* (TPP1), *DKC1* and *ERCC1*.

Conclusions: Our findings suggest that expression level of *ACD*, *DKC1* and *ERCC1* may serve as early markers of carcinogenesis in colon cancer. The results were obtained by the applications of statistical analysis of semi-quantitative microarrays method alone. They should thus be regarded as preliminary and need to be confirmed in part II of our study and in further trials of larger populations.

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Mechanism underlining tumourigenic genetic changes

Association of *ERBB2* gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients

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Abstract

Poster presentation

ERBB2 gene amplification and overexpression in breast cancer is a prognostic and predictive factor and also a therapeutic target. In gastric cancer, there are some studies evaluating ERBB2 overexpression/amplification, but its clinical significance remains unclear. In this study, we evaluated ERBB2 status in 463 gastric carcinomas using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) and compared the findings with clinico-pathological characteristics and with disease-specific survival. Among the 463 gastric carcinomas, 43 (9.3%) showed ERBB2 overexpression by IHC and 38 (8.2%) showed *ERBB2* amplification by FISH. Perfect IHC/FISH correlation was found for the 19 cases scored as 0 (all negative by FISH) and also for the 25 cases scored as 3+ (all positive by FISH). One out of six carcinomas scored as 1+ and 12 out of 18 carcinomas scored as 2+ were positive by FISH. *ERBB2* amplification was found in both histological components of two mixed (F. Carneiro) carcinomas, suggesting that it occured prior to other genetic and/or epigenetic events that lead to phenotypical divergence. Interestingly, we have found *ERBB2* amplification in two early gastric carcinomas, strengthening the hypothesis that this is an event that takes place early in gastric carcinogenesis. *ERBB2* amplification was more common in intestinal type carcinomas ($p = 0.007$) and in carcinomas with expansive growth ($p = 0.021$). *ERBB2* amplification was associated with a trend towards worse survival for patients with intestinal ($p = 0.268$) and lymph node negative ($p = 0.153$) gastric carcinomas. A statistically significant association was found between *ERBB2* amplification and worse survival in patients with expansive gastric carcinomas ($p = 0.014$). These data suggests that *ERBB2* amplification confers increased malignancy to carcinomas deemed less aggressive based on growth pattern. Venous invasion assessed through orcein staining of tissue sections was found to be a marker for worse prognosis ($P < 0.001$), but no association was found between venous invasion and *ERBB2* amplification.By studying the largest series of gastric cancer patients for ERBB2 amplification/overexpression, we conclude that ERBB2 status may have clinical significance in subsets of patients and that further studies are warranted to evaluate whether gastric cancer patients whose tumors present *ERBB2* amplification may benefit from targeted therapy with trastuzumab.

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11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours

Bilbao, Spain, September 6-9, 2008

Mechanism underlining tumourigenic genetic changes

Hereditary multiple basal cell at young age in patients without germline PTCH mutations

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Abstract

Poster presentation

Basal cell carcinoma (BCC) of the skin is one of the most common malignancies in elderly people in Western countries. Early-onset BCC is also common in different syndromes, including Gorlin syndrome (also known as nevoid basal cell carcinoma syndrome), which is characterized by germline mutations in the PTCH1 gene on 9p22.3. However, a considerable proportion of the multiple BCC patients diagnosed at young age remain genetically unexplained.

In the present study, we performed high-resolution genomic profiling on normal tissue DNA in patients diagnosed with multiple BCCs below 40 years of age, and without germline mutations in PTCH1. In one of these patients we identified a genomic deletion of 1.3 Mb in size, encompassing 4 protein-coding genes. In one of these genes, encoding a cell cycle regulator, second hit somatic mutations were identified in the remaining allele in tumor DNA from this patient. Interestingly, normal keratinocytes isolated from this patient showed an abnormal in vitro growth pattern as compared to those from control individuals, suggesting that mono-allelic loss of this gene already affects keratinocytic cell division. Currently, we are screening larger cohorts of PTCH-negative multiple BCC patients to elucidate the incidence of this novel BCC predisposing gene. The results of this screen will be presented.

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Mechanism underlining tumourigenic genetic changes

Comparative genomic hybridization and cytogenetic studies on the genomic imbalances in the well-differentiated and dedifferentiated liposarcomas

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Abstract

Poster presentation

Well-differentiated liposarcomas (WDLPS) are classified as a local aggressive group of LPS and account for about 45% of all LPS. The second subtype of these sarcomas is dedifferentiated liposarcomas (DDLPS), which represent malignant tumors and about 90% of these tumors arise de novo, whereas 10% are recurrences of WDLPS. Cytogenetically, both subtypes of sarcomas are characterized by the presence of ring chromosomes (r) and/or giant marker chromosomes (giant mar). Amplification of chromosome region 12q11/13-q21/24 has been repeatedly established with CGH studies.
The study included 22 WDLPS and 15 DDLPS tumors. CGH and cytogenetic studies were performed on fresh tumor samples. Chromosomes were obtained from the primary tissue cultures. DNA was isolated from the frozen tissue samples. The integrated CGH and cytogenetic studies confirmed characteristic chromosome changes in both subtypes of liposarcoma, especially the amplification of region 12q13-22. We have shown that amplifications of 12q13-21 in WDLPS tumors and 12q13 and 12q14-22 in DDLPS tumors are correlated with shorter overall survival of the patients ($p=0.003$, $p=0.004$ and $p=0.004$, respectively). In the WDLPS tumors, the main genomic imbalances were formed by additional copies of genetic material (in 62% of tumors no losses were found), whereas in DDLPS tumors both gains and losses were observed. Amplification of chromosome 14q and additional copies of chromosome 5p were found exclusively in WDLPS tumors, whereas amplification of chromosome 5q appeared in DDLPS tumors only. Additional copies of chromosome 5p14-15 in WDLPS tumors and chromosome 5q15-q23 in DDLPS tumors displayed significant correlation with fatal disease outcome ($p=0.012$ and $p=0.047$ in multivariate Cox analysis, respectively).
Our results pointed out the differences in genome balance between WDLPS and DDLPS liposarcomas which may play a role in biological and clinical differences between both tumor subtypes.

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Mechanism underlining tumourigenic genetic changes

Analysis of epidermal growth factor receptor gene copy number in glioblastomas and its relation with the protein expression

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Abstract

Poster presentation

Glioblastoma multiforme is the most frequent and most malignant brain tumor. It manifests itself preferentially in adults, with a peak incidence between 45 and 70 years old. Despite progress in surgical and adjuvant therapy, the mean survival of patients with this neoplasm is still around one year. Heterogeneous pathological features are associated with complex molecular heterogeneity. Varying genetic alterations lead to development of malignant phenotype expression in glioblastoma. Loss of heterozygosity 10q is the most frequent genetic alteration, followed by amplification of the epidermal growth factor receptor gene (*EGFR*), *TP53* mutations, *INK4a* homozygous deletion and *PTEN* mutations. Epidermal growth factor receptor overexpression occurs in a significant percentage of cases of glioblastoma multiforme, and amplification has been found in approximately 40% of these neoplasms. Fluorescence in situ hybridization (FISH) is a powerful methodology to detect *EGFR* copy number abnormalities, together with real time PCR We performed FISH analysis using EGFR probe in cultured cells and paraffin sections. EGFR gene amplification/overexpression was determined by RT-PCR and inmmunohistochemical studies using EGFRvIII, in 40 cases of glioblastomas. The 42% of cases had *EGFR* amplification displayed multiple spots suggesting the pattern of double-minute chromosome. In 13 cases of them, the amplification was present in 50%-100% of cells and in 4 cases in 5%-50% of cells. The 25% of cases showed a low number of signals for each chromosome 7, appearing in isolated cells or in 10%-70% of tumoral cells. Trisomy/polysomy of chromosome 7 was found in 70% of cases; in 83% of cases with high amplification of *EGFR*, in 70% of cases with low amplification, and in 53% of cases without amplification. The EGFR gene dosage by q-PCR analysis varied between 1.9 and 227.8 copies. The amplification of *EGFR* in glioblastomas is characterized for an important heterogeneity. This event has been correlated with protein expression and clinicopathological characteristics.

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Mechanism underlining tumourigenic genetic changes

Chromosomal imbalances, metabolic (HR-MAS) and gene expression profiles in bening and atypical meningiomas

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Abstract

Poster presentation

Meningiomas are usually benign tumors (grade I) of the Central Nervous System; however, they can recur after surgical resection and occasionally show histological progression to a higher malignancy grade II and III The second most frequently reported genetic abnormality after 22q loss is deletion of 1p, although alterations in 9q, 10q, and 14q are also involved in meningioma progression. In contrast to these genetic alterations, relatively little is known about the changes at the transcript level that are associated with the different meningioma malignancy grades.

Likewise, high resolution magic angle spinning (HR-MAS) MR spectroscopy of human tissue provides high metabolic detail of unprocessed tissue samples, and has recently been used in the study of these tumours. One hundred and twenty tumors comprising 72 benign, 34 atypical, and 14 malignant meningiomas were examined by means of cytogenetic and fluorescence in situ hybridization analysis. To better understand the molecular basis of meningioma progression, we performed microarray-based expression profiling and metabolic profiles of 30 meningiomas of different malignancy grades. The cytogenetic and FISH results showed monosomies and chromosomal imbalances affecting chromosomes 1, 4, 7, 10, 14, 18 and 22, principally in atypical and malignat meningiomas. The chromosome 1 is implicated at 1p11, 1p13, 1p21, 1p22, 1p32, and 1q21 breakpoints. All but one of the cases with simultaneous 1p deletion and alterations of chromosome 14 were grade II and grade III; all the grade III cases were recurrent. These results support the possible association between changes in 1p and chromosome 14 with the evolution of aggressive meningiomas through tumor progression. Our first approach in the gene expression profile was performed using the whole data included in the array using non supervised statistical analyses (PCA and hierarchical clustering). Then we increased the complexity of analysis following two different approaches: we selected genes according to biological functions involved in tumor progression, and genes included in chromosomes 1p, 6q, 10, 14q.

Several conclusions were extracted from our analysis. We found a poor correlation between location and histological type. Benign meningiomas (grade I) conform a more homogeneous group than atypical tumors or anaplastic tumors. Finally we selected a matrix of genes involved in different biological functions (differentiation, cell signaling, cellular cycle, apoptosis, structural components of cellular matrix, and development) which were useful to distinguish and discriminate atypical and benign meningiomas within our samples. Metabolic differences between meningioma grades include changes in the levels of glutathione. Glutathione role in cancer is still unclear, as it may act both as protective and pathogenic factor. Glutamine and

glutamate, which are related to glutathione metabolism and have been associated with tumor recurrence, are also increased in atypical meningiomas. Other metabolites associated with tumour malignancy that show statistically significant differences between benign and atypical meningiomas include phosphocholine and phosphoethanolamine.
Overall, this work indicates that the additional information obtained by NMR metabolomics applied to biopsies of human meningiomas may be useful for assessing tumor grade and determining optimum treatment strategies.

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SESSION

Cancer cell biology

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Cancer cell biology

Molecular cytogenetic characterization of tumour initiating cells

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Abstract

Oral presentation

Cancers are notorious for their ability to survive treatment and recur. Despite the clonal origin of many cancers, a notable characteristic of primary tumors is a marked degree of cellular heterogeneity. There is direct indication of a tumor-initiating or progenitor cell origin for at least some solid tumors: glioblastoma, breast cancer, colon carcinoma, melanoma, prostatic carcinoma and ovarian cancer. However, little is known on the genomic characterization of putative tumor initiating cells and their genetic stability. A thorough chromosomal and genotypic characterization of stem-like tumor cells is required to truly understand how closely they fulfill the criteria of cancer stem cells. We isolated and propagated cells cultures growing as spheres in serum-free medium from 6 established epithelial cell lines (A549, MCF7, 734B, SKOV3, BT474, JR8) and three primary glioblastoma surgical specimens. Spheres and adherent cells were analyzed for stem-like properties including sphere-forming efficiency, expression of phenotypic markers and tumorigenisis in vivo. A detailed molecular-cytogenetic characterization (using Spectral Karyotyping Imaging, SKY, and fluorescent in-situ hybridization, FISH) of sphere-growing stem-like cancer cells and serum-cultured adherent cells was performed. Overall, SKY analyses showed that sphere-growing cells, both from primary tumors and long-term cell lines, possess a more rearranged karyotype then adherent cells and display additional and more complex chromosomal rearrangements with clear marks of karyotypic evolution. Moreover, a certain degree of genetic instability was observed during in vitro propagation. Overall, these results suggest the existence of heterogeneous, de-differentiated and genetically unstable subpopulations of cells within cell-lines and tumors showing markers of karyotypic evolution. These populations, due to their genetic instability, are endowed of an intrinsic ability to generate highly tumorigenic cells with stem-like features.

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Cancer cell biology

Characterization of spontaneously transformed murine epithelial cells

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Abstract

Oral presentation

The cellular phenotypes of cells undergoing transformation have been extensively studied, predominantly in fibroblasts; however, the precise genomic changes that initiate immortalization and ultimately tumorigenesis have yet to be elucidated. In this study, 42 primary epithelial cell cultures, derived from bladder, cervix, colon, kidney, lung, mammary glands were established from C57Bl6 mice and became spontaneously transformed, i.e., no chemical, viral or genetic manipulations in vitro. Morphological changes, centrosomes (numbers and distribution), telomerase enzyme activity, chromosome complements, genomic imbalances as measured by CGH/aCGH, and RNA gene expression levels were monitored throughout the transformation process.

We selected three time points referred to herein as: pre-immortal, immortal, and transformation stages. Our analyses revealed that extensive centrosome instability, formation of bi-nucleate cells, polyploidy, and chromosome aneuploidy all preceded transformation. SKY analysis revealed recurrent and non-random chromosome gains and losses, including partial or complete loss of MMU4 (harboring the tumor suppressor gene p16/Ink4a/Cdkn2a) in 100% of the pre-immortal cultures. Additional transformation-specific rather than tissue-specific imbalances consisted of gains of chromosomes MMU2, 5, 10, 15, 19, and losses of MMU9, 12, 16, and Y. FISH analysis detected amplifications (e.g., Myc and Mdm2) in the form of double minutes (Dmin) in 4 bladder, 3 kidney, and one mammary cell line. Telomerase activity was assessed (TRAPP assay) for normal epithelial cells and two time points (immortal, transformed) for multiple cell lines, revealing variable levels among all tissue types. Interestingly, 2 bladder cell lines exhibited 100-200 fold increase of telomerase relative to normal cells and negative controls. Twelve transformed cultures (5 bladder, 5 kidney, 2 mammary) were tested for tumorigenicity using the nude mouse assay (3 mice/cell line). Two cell lines in particular, expressing both high telomerase activity, and Myc amplifications, formed tumors within one week, as did another bladder cell line with low telomerase, and Mdm2 amplifications. One kidney culture, in particular containing amplifications of a chromosomal region derived from MMU2 also yielded tumors rapidly. The tumorigenic potential of the cell lines did not always correlate with high telomerase or genomic instability. Array CGH refined regions of genomic imbalances found by SKY, subsequently confirmed with RNA gene expression microarrays. Numerous genes are deregulated during spontaneous transformation, many of which are commonly up-regulated or down-regulated in human bladder and kidney cancers.

This is the first multi-disciplinary analysis of both the phenotypic and genomic changes prevalent in the spontaneous transformation process of murine epithelial cells, derived from six different tissue types. Furthermore, we have demonstrated that chromosome aneuploidy, gene amplifications and centrosome instability always precedes transformation but do not necessarily predicate tumorigenic potential, and increased telomerase activity is not a requirement for tumor formation.

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Cancer cell biology

Genomic alterations and expression profiling in uveal melanoma

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Abstract

Oral presentation

Uveal melanoma (UM) is the most common primary intraocular malignancy in the western world with an annual incidence of 7 per million. Approximately 50% of the patients die eventually due to metastatic disease, most commonly located in the liver. A number of clinical and histopathological parameters have been associated with development of metastases. Also cytogenetical prognostic factors are reported. Cytogenetically these tumours are characterized by typical chromosomal losses and gains, such as loss of chromosome 1p, 3, 6q and gain of chromosome 6p and 8q.

Here we assess the independent value of numerical changes in a large series of UM Furthermore, to gain insight in the genes responsible for poor prognosis, we have applied gene expression profiling on a selected number of tumours with extensive clinical, histopathological, cytogenetic and follow-up data.

Patients and methods: 120 tumours from UM patients were analysed for numerical changes of chromosome 1, 3, 6 and 8 with cytogenetic analysis, fluorescent in situ hybridisation and/or comparative genomic hybridisation. Data were correlated with disease outcome in univariate and multivariate analysis using Kaplan-Meier and Cox regression analysis. Gene expression profiles of 46 out of the 120 UMs were obtained using Affymetix gene chips. Data was analysed with Omniviz and PAM software and validated with real-time PCR Furthermore, a locally adaptive statistical procedure (LAP analysis) was performed to identify differentially expressed chromosomal regions.

Results: At a mean follow up time of 45 months, 42 patients had died or were suffering from metastatic disease. In the multivariate analysis, the effect of monosomy 3 on survival was largely modified by changes in chromosome 1p36. We found that regarding all chromosomal variations that concurrent loss of chromosome 1p36 and 3 is an independent prognostic parameter for disease free survival ($p<0.001$). On basis of micro-array expression data the primary UMs could be classified in to two distinct molecular classes with a strong prognostic value ($p<0.001$). By combining both data sets using locally adaptive statistical procedure two regions on the chromosome 3p arm with decreased expression in tumours with a decreased disease free survival could be identified. The identified regions with lower expression on the chromosome 3p arm could harbour genes responsible for the poor prognosis.

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Cancer cell biology

Relationships among truncating mutations, genomic instability and cancer risk in a molecularly defined Rothmund Thomson case

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Abstract

Poster presentation

Rothmund-Thomson syndrome (RTS; MIM #268400) is a rare autosomal recessive genodermatosis characterized by poikiloderma; sparse hair, eyelashes, and/or eyebrows/lashes; small stature; skeletal and dental abnormalities; cataracts (RTS type I); and increased risk for cancer, especially osteosarcoma (RTS type II). Mutations in the *RECQL4* (MIM #603780) gene, a member of the RecQ helicase family together with those responsible for Werner (*WRN*) and Bloom (*BML*) chromosomal instability syndromes, account for about 66% of RTS patients. Molecular mechanisms underlying the cancer predisposition in RTS II patients are not fully understood and no diagnostic tests are nowadays available to assess genomic instability. Moreover the lack of a clear genotype-phenotype correlation does not allow to identify RTS patients with increased cancer risk. Here we report the case of a four-years-old RTS patient without cancer carrying two apparently truncating mutations: the yet undescribed stop mutation at exon 14 (R758X) and the reported frameshift mutation g.4644_4645delAT at exon 15, leading to a stop codon 51 residues downstream. As it has been proposed that the presence of at least one truncating mutation is associated to increased cancer risk in RTS patients, we investigated the potential effect of both RECQL4 inactivating mutations at mRNA level. Transcript analysis by semi quantitative RT-PCR and cloning revealed that the mRNAs harboring the mutations are not degraded. Moreover, the stop mutation seems to enhance usage of a never described physiologic splice site predicted to cause an in-frame loss of 66 amino acid at the protein level. The functional significance of this physiologic isoform, detected also in normal controls, is unknown. The frameshift mutation, besides the expected isoform, yield aberrant spliced transcript which is predicted to cause an in-frame loss of 74 amino acid at protein level. Here we demonstrate that characterization of *RECQL4* mutations needs more extensive evaluation of physiological and abnormal transcripts, because a few truncating mutations apparently leading to a complete loss of function may preserve some RECQL4 protein activity acting on splicing. This represents a crucial step for the establishment of a correct genotype-phenotype correlation and cancer risk evaluation. Secondly, we investigated whether the identified mutations mark some kind of genomic instability by applying the cytokinesis-block micronucleous (CBMN) assay (a comprehensive tool normally used for quantifying instability in cancer cells) on lymphoblastoid cell lines from the patient and her parents. Results showed a spontaneous marked increase in chromosome instability in the patient (13,7% of abnormal binucleated cells) relative to normal controls (mean of 5.75% of abnormal binucleated cells). Parents values were similar to those of normal controls (5,7% and 5,1%). Even if further studies are needed to assess the relationship between genomic instability detected by CBMN assay and cancer risk, our results indicate that this test may represent a useful tool for detecting biomarkers of genome damage and for predicting cancer risk in clinical practice.

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SESSION

Epigenetic changes in solid tumours

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Epigenetic changes in solid tumours

The role of telomere length in telomerase based anticancer therapies

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Abstract

Oral presentation

Telomeres protect chromosomes from attrition, irregular recombination and end-to-end fusions, and are responsible of proliferative senescence. Continuous proliferative telomere attrition leads to critical telomere length that in turn activates the DNA Damage Response mechanism (DDR), unleashing cellular senescence. Telomerase, a retrotranscriptasa riboprotein complex prevents cell cycle related telomere attrition by adding sequences of the microsatellite TTAGGG at the end of single stranded telomeres. This mechanism, a protective effect in highly proliferative cells, is pathologically activated in genomically unstable cancer cells to become immortal. It has been postulated that a single, critically short telomere in the cell may be responsible for triggering the protective effect of telomerase. This seems to be the corollary of several recent studies on telomerase inhibition and vaccination as cancer treatment. Confirming this hypothesis would be very relevant to understand the mechanism and improve the efficiency of telomere-related anticancer therapies. This requires being able to identify and measure the shortest telomere of the cell in situ, knowing the telomerase expression status of the cell. We have developed a staining protocol that permits simultaneous immunofluorescence of the human telomerase catalytic subunit (hTERT) and fluorescent in situ hybridization of the telomeres (Telo-FISH). This, along with novel image analysis tools, allows us to simultaneously measure telomere length and the expression and spatial distribution of hTERT in intact cells. This way, we can determine cell-by-cell the relationship between expression of hTERT and the telomere length maintenance in both cancer and normal cells. Our image analysis protocol proceeds in four steps: epifluorescence 3D image acquisition, deconvolution, linear unmixing and image segmentation, and allows us to quantify the length of every telomere in an absolute way. To that end, our telomere quantification measurements are calibrated by correlation with a molecular weight provided by the Telomere Restriction Fragment method (TRF). We have used our methods to study a small airway epithelial cell line (SAEC), a normal epithelial cell line immortalized with CdK4 and hTERT and three well established lung tumour cell lines (A549, H1299 and H157). In all these cell lines, we have measured, cell by cell the length of the shortest telomere of the cells, the expression of hTERT and the effects of a telomerase inhibitor (MST-312). Our results indicates that hTERT is preferentially deployed nearly the shortest telomeres of the cell, and that this correlation increases as telomeres shorten with time. Preliminary results on the inhibitory effect of MST-312 seem to indicate that it is the relative length of the shortest telomere of the cell, and not the average telomere length what elicits the activity of telomerase and the inhibitory effect of MST-312. In clinical practice, should these preliminary results be confirmed, these data suggest that an in situ quantification of telomere length in cancer biopsies could determine the possible outcome of a telomerase inhibition anticancer therapy.

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Epigenic changes in solid tumours

Genome reorganization during invasive cell growth

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Abstract

Oral presentation

Invasive cell growth is a physiological process executed by stem and progenitor cells during liver embryonic development and postnatal organ regeneration. Interestingly, this program is usurped by cancer cells during metastasis. Growing evidence indicates that integrins are responsible for the invasive capacity of progenitor and cancer cells, enabling them to survive and proliferate in foreign microenvironments. However, it is not known how the extracellular signals sensed by integrins are converted into responses at the genomic level.

Here, we assessed the genomic events that occur during the invasive growth of hepatic progenitor cells elicited by β1 integrins-emanating signals. To assess specific signalling stimuli in MLP29 cells, we used an antibody against the α5β1 integrin, the main fibronectin (FN) receptor in hepatocytes. Functional blocking of α5β1 induced actin cytoskeleton reorganization and a phenomenon of cell spreading similar to that observed during invasion.To investigate genome organization at a global level, we performed 3D-FISH with a pan-centromeric probe after α5β1 functional blockade, as well as upon activation with FN. We found that the number of chromocenters (clusters of centromeres) increase along with a decrease in their volume following α5β1 functional blockade. Conversely, less, but larger, chromocenters were detected after activation of this integrin, all without changes in the volume of the cell nucleus. These α5β1-mediated effects were further proved by the observation that the α5β1 blocking antibody drastically increased the level of acetylated histone H3 K9/14 in MLP29 cells. Furthermore, gene expression profiling revealed that such nuclear architecture remodelling is associated with functional changes of the genome. Interestingly, classification of the differentially expressed genes (DEGs) by gene ontology revealed that cell spreading correlates with up-regulation of genes belonging to the nuclear structure and nucleic acid binding proteins categories. Moreover, we detect that not all chromosomes are equally affected: In fact, more DEGs were found to map to chromosomes 2, 3, 5, 7, 8 and 11.

Collectively, these results suggest that invasive growth in hepatic progenitor cells is, at least in part, mediated by the activation or functional blockade of α5β1 integrin, involving remodelling of the nuclear architecture associated with apparently selective functional changes of the genome.

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Epigenic changes in solid tumours

The epigenetics of human synovial sarcoma: towards novel therapeutic strategies

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Abstract

Oral presentation

Synovial sarcomas (SS) are aggressive soft-tissue tumors which mainly affect children and young adults and account for up to 10% of all human sarcomas. In over 95% of all cases, the SS18 gene (previously called SYT or SSXT) is fused to either one of three highly homologous SSX genes (SSX1, SSX2 or SSX4). Since the SS18 and SSX proteins are involved in opposite epigenetic regulation activities, we hypothesize that the SS18-SSX fusion proteins act as activator-repressors of transcription, probably in an epigenetic fashion (de Bruijn et al 2007). Consistent with this notion, it has recently been shown that epigenetic drugs such as histone de-acetylase (HDAC) inhibitors exhibit a remarkable efficacy in the inhibition of in vitro and in vivo growth capacities of synovial sarcoma cells. Through microarray-based profiling we found that the SS18-SSX fusion protein affects the expression of a specific set of target genes, including IGF2, CD44 and EGR1 (de Bruijn et al 2006). The EGR1 gene, which has been proposed to function as a tumor suppressor, is repressed in primary synovial sarcomas and in cell lines expressing the SS18-SSX fusion gene. By chromatin immunoprecipitation (ChIP), we found that the SS18-SSX fusion protein is recruited to the EGR1 promoter. Our ChIP data also demonstrated that the EGR1 gene is repressed by the Polycomb silencing complex. Finally, we found that HDAC inhibitor-induced growth arrest of synovial sarcoma cells is associated with up-regulation of the EGR1 gene and abrogation of its Polycomb-mediated repression. Taken together, our data indicate that the SS18-SSX fusion protein affects downstream target gene expression via epigenetic promoter modification. HDAC inhibitors effectively reverse this SS18-SSX induced effect which leads to re-activation of tumor suppressors such as EGR1. This finding may explain the observed efficacy of HDAC inhibitors on the growth of synovial sarcoma cells and opens up new avenues for the treatment of this aggressive soft tissue tumor.

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Unbiased differential methylation screening assay for applications in cancer epigenetic research

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Abstract

Oral presentation

The spectrum of differential methylation screening methods is vast, and among the most well known are methylation sensitive restriction landmark genomic scanning, methylation sensitive representational difference analysis, methylation sensitive arbitrarily primed PCR, amplification of intermethylated sites (AIMS), differential methylation microarrays, gene expression analysis following DNA demethylation, methyl-CpG immunoprecipitation. Still, none of these fulfil all of the requirements to turn it into a universal unbiased differential methylation screening method. We suggest that these minimal and sufficient requirements are: unbiased nature of screening, high reproducibility and an option to standardize the experimental results, fast and easy handling, costeffectiveness, fast and easy mapping of the identified differentially methylated DNA fragments, and viability for parallel analysis of unlimited number of samples. In an effort to develop a synthetic unbiased screening technique to serve as a possible basis for cancer epigenome research we adopted AIMS as a method, which is in best agreement with the above requirements. It should be noted though, that, with high reproducibility and relative simplicity AIMS possesses serious disadvantages as to fragments resolution and mapping of the identified differentially methylated loci. As far as AIMS generates significant numbers of PCR products, the mode of DNA fragments detection has to be optimised to achieve appropriate resolution and easy mapping to the genome. Single-nucleotide resolution would be preferable, and an obvious way to achieve this is capillary electrophoresis (CE). Elaboration of CE also makes it unnecessary to label PCR primers radioactively as far as the very detection method relies on fluorescence. Fluorescent labelling in conjunction with CE technique has other advantages: there is no further need to use slab gels, and the detection becomes simultaneous, more reliable and easily archived and annotated. Moreover, representations of compared genomes may be labelled with different fluorescent dies and thus be analysed in one and the same capillary, which provides supreme comparison accuracy. At the same time CE does not generally provide preparative option, thus sequencing of the bands in order to identify the genomic localization of fragments has to be substituted by another approach, which is advised by the very high resolution of CE DNA analysis. Knowledge of exact nucleotide length of the predicted and practically obtained AIMS product allows its in silico identification in genomic context. In order to utilize this option we have designed specific software for automatic identification of differentially methylated fragments as well as the software capable of identifying the AIMS fragments in silico by alignment to available genome databases. Additional computer service is available to assist in experimentation design respective to specific applications. With technical variations this approach may be elaborated to identify novel targets of DNA methylation/demethylation as well as to characterize epigenomic status as a whole. We suggest that the assay is universal and can be applied for differential methylation screening in any disease where epigenetic component may be suspected.

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Epigenetic changes in solid tumours

Differential positioning of gene markers in normal and malignant tissues

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Abstract

Oral presentation

The genome is non-randomly organized within the three-dimensional space of the cell nucleus. The nuclear position of many genes and genomic regions changes during physiological processes such as proliferation, differentiation and, importantly, disease.
We hypothesize that we can exploit the changes in gene positioning patterns as indicators of disease. To this end, we have analyzed the spatial position of a defined set of cancer-associated genes in an established mammary epithelial 3D cell culture model of early stages of breast cancer. We find that the genome is globally reorganized during normal and tumorigenic epithelial differentiation. Systematic mapping of changes in spatial positioning of cancer-associated genes reveals gene specific positioning behavior and we identify several genes which are specifically repositioned during tumorigenesis. Alterations of spatial positioning patterns during differentiation and tumorigenesis were unrelated to gene activity.
Our results demonstrate the existence of activity-independent genome repositioning events in early stages of tumor formation.

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Epigenetic changes in solid tumours

Similar chromosomal expression profiles of genetically favourable and senescent neuroblastoma cells: signs for a common epigenetic pattern?

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Abstract

Poster presentation

The tumour regression phenomenon found in patients with localized or 4s neuroblastoma (NB) is linked to a specific genomic pattern: absence of *MYCN* amplification, a near-triploid DNA content and absence of segmental chromosomal aberrations. However, the molecular mechanisms underlying the spontaneous regression process are still unknown. Another pathway leading to a non aggressive NB phenotype *in vitro* is the phenomenon of cellular senescence found in *MYCN* amplified neuroblastoma cell lines which have expulsed the amplified gene during the senescence process. Comparative expressed sequence hybridization (CESH) was applied to visualize the global chromosomal expression profile in both cell types. The expression profiles of genetically favourable NBs (n=3) and those of senescent NB cell lines (n=4) were compared. In both instances non senescent, *MYCN* amplified, NB cell lines were used as reference. The chromosomal hybridization pattern visualized by CESH displayed global, genome-wide transcriptional differences on the target chromosomes covering the entire human genome in unbiased fashion. Genetically favourable tumours displayed a uniform expression pattern. High expression was observed predominantly in AT rich chromatin, while the aggressive NBs displayed high expression at different loci (including 2p23-24, i.e. MYCN locus) and in predominantly GC rich chromatin fractions. Surprisingly, when comparing the chromosomal expression profile of genetically favourable NBs with those of senescent NB cells, a similar expression pattern was found in both cell types. Despite the fact that the favourable and senescent NB cells are very different concerning their genesis, the global chromosomal expression profiles are similar. Epigenetic mechanisms are likely to be responsible for these global expression changes in senescent cells.

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SESSION

Technological advances

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Technological advances

HAPPY Mapping: a flexible method for examination of genomic rearrangements in cancer genomes

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Abstract

Oral presentation

The importance of chromosomal translocations in the development of epithelial cancers has recently emerged with the identification of fusion genes in different carcinomas. The most significant example has been identified in prostate cancer where approximately 70% of cases have gene fusions between ETS transcription factor genes to the TMPRSS2 gene as a result of translocation or deletion (Tomlins et al., 2005). In order to identify breakpoints we need to employ technologies used to map the human genome. HAPPY mapping (mapping based on the analysis of approximately HAPloid DNA samples using the PolYmerase chain reaction) is a means of making accurate maps of genomes, which has been applied to a wide range of species (Dear & Cook, 1989). It has enormous potential for mapping structural changes in cancer genomes, such as deletions, inversions and translocations. Inversions and other balanced rearrangements are difficult to identify by other currently available methods. The flexibility of this technique allows analysis of any region of the genome and at any resolution, so that mapping a breakpoint can be refined to a clonable distance.

As a proof of principle experiment we show here the confirmation of a balanced translocation between chromosome 1 and chromosome 8 in a highly rearranged breast cancer cell line HCC1187 using markers spaced at 7kb intervals. A rearrangement in another breast line, T-47D, initially mapped using FISH, has also been HAPPY mapped to 38kb resolution, and turned out to be much more complex than initially thought. We confirm HAPPY mapping is a viable technique that can be used to examine rearranged cancer genomes.

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Technological advances

Identification of transcriptional targets by ChIP-Sequencing in t(X;1)-positive renal cell carcinomas

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Abstract

Oral presentation

Previously, we and others showed that in a subset of human renal cell carcinomas the bHLH-LZ transcription factor TFE3 is recurrently fused to a novel protein designated PRCC. Subsequently, we established that the resulting PRCCTFE3 fusion product acts as an oncogenic protein, both *in vitro* and *in vivo*. In addition, we found that PRCCTFE3 acts as a more potent transcriptional activator than wild-type TFE3. More recently, a functional cDNA screen revealed that TFE3 over-expression renders cells insensitive to the anti-proliferative effects of the G1/S cell cycle regulator pRB. We propose that also the PRCCTFE3 fusion protein may act through a cell cycle-mediated deregulation of proliferation. In order to identify downstream transcriptional targets of the PRCCTFE3 fusion protein, we initiated to use chromatin immunoprecipitation (ChIP). Specifically, we are employing a recently developed variant of this technology, called ChIP-Sequencing, which combines ChIP with massive parallel sequencing to identify and quantify in vivo protein-DNA interactions on a genome-wide scale. The identification of novel PRCCTFE3 transcriptional targets and its implications for our understanding of the role of cell cycle (de-) regulation in renal tumor development will be discussed.

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Technological advances

Identification of genes harboring nonsense mutations

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Abstract

Oral presentation

According to the classic Knudson two hit model the biallelic inactivation of (a) tumor suppressor gene(s) is a crucial step in the development of cancer. In fact it was this very model that initiated a hunt for members of this class of genes. These searches have predominantly been based on LOH approaches, trying to identify so-called "smallest regions of overlapping deletions (SRO's)". Often, these SRO's turned out to be relatively large, encompassing many genes. Therefore the search for tumor suppressor genes would highly benefit from a genome wide mutation screening method that could be used in parallel to the LOH approaches. Noensi et al (2001) designed a genome wide screening method to identify genes harbouring truncating mutations, called GINI (Gene Identification by NMD Inhibition). The transcripts of these genes are targets for the NMD (Nonsense mediated MRNA Decay) pathway and are degraded during a so-called "pioneer round of translation". In the GINI approach, this pathway is (pharmacologically) blocked. The resulting accumulation of nonsense transcripts can be detected using gene expression microarrays and confirmed by RT-PCR This approach has been used (in modified versions) by several groups to identify mutated genes in cancer cell lines. All methods deal with the same problem: inhibition of the NMD pathway causes a stress response, resulting in false positives. We have developed a method that makes it possible to obtain a selective list of nonsense transcripts for each individual (cancer) cell line by combining three methods of inhibiting the NMD pathway: 1) by using siRNA against Rent1, a crucial protein in the NMD-pathway, 2) by emetine, which inhibits the movement of the ribosome along the mRNA, and 3) by caffeine, which inhibits the phosphorylation of Rent 1 by SMG-1. Inhibiting the NMD pathway in these different ways presumably causes the upregulation of different stress response genes. Thus, genes upregulated by all three methods are selected for subsequent analysis. We will present the data obtained with two prostate cancer cell lines harboring known nonsense mutations and show that indeed combining the three methods gives an enormous reduction of candidate genes. Further we hope to present some results of our GINI-method on clear cell renal cell carcinoma cell lines.

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Technological advances

Array CGH after FISH-MD reveals that typical marker chromosomes in ovarian cancer frequently show fusions between 11q13 and 19p13.3

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Oral presentation

By SKY analysis we identified different marker chromosomes in ovarian cancer cells. One of them, a translocation product of chromosomes 11 and 19 with unknown breakpoints, has been ascertainable in different primary cultures and cell lines of ovarian cancer. We micro-dissected the chromosome with the FISH-MD method to delineate the breakpoint of this recurrent marker chromosome. This was done on FISH labelled metaphase spreads of the SKOV3 and an ascites ovarian cancer cell line named AON established in our laboratory. Hybridization with our FISH-MD probes, amplified by DOP-PCR, on different DNA chips (Array painting) revealed an almost identical fusion region of the isolated marker chromosome of the two cell lines. The utilized sub-megabase resolution 32k tiling path BAC array as well as the 244k oligo-array from Agilent permitted the containment of the fusion region on chromosome 19 to 107 kb and on chromosome 11 to 76 kb. While the BAC-tilling array indicated complete signals in represented areas, the Agilent 244 k-array only showed a signal in every 16th oligo with an average distance of 97kb. However, according to the results obtained so far we could design a customized Agilent high density oligo-array with an average oligo distance of 170 bp in this fusion region. Simultaneously, the existence of a marker chromosome containing a fusion between 11q13.1 and 19p13.13 or around both regions could be confirmed by interphase FISH with probes of selected BAC-and Fosmid-clones in 16 out of 32 primary ovarian cancer cell cultures.

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Technological advances

Design of split-signal FISH probe for the detection of TFE3 translocations in Xp-translocation Renal Cell Carcinoma (RCC)

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Abstract

Poster presentation

The 2004 WHO classification for RCC recognizes several distinct subtypes of RCC based on morphologic features and genetics. This classification includes a recently recognized family of Xp translocation RCCs. Xp translocation RCC is associated with rare and juvenile forms of the malignancy. These tumors are characterized by various translocations involving chromosome Xp11.2, all of them resulting in gene fusions that affect the transcription factor TFE3 gene. Five distinct gene fusions involving TFE3 have been characterized: ASPL-TFE3 or t(X;17)(p11.2;q25); PRCC-TFE3 or t(X;1)(p11.2;q21); PSF-TFE3 or t(X;1)(p11.2;p34); NoNo-TFE3 or inv(X)(p11q12) and CLTC-TFE3 or t(X;17)(p11.2;q23). A total of 48 cases have been retrieved from the Mitelman Database of Chromosome Aberrations in Cancer. The most distinctive immunohistochemical feature of these neoplasms is nuclear labeling for the TFE3 protein using an antibody that recognizes their C-terminal portion, which is retained in all of the reported fusion products.

The aim of this study was to develop a genetic tool that allows the detection of tumors with translocations that involve the TFE3 gene. A split-signal FISH probe has been designed to detect TFE3 translocations independently of the involved partner gene using labeled BACs (Bacterial Artificial Chromosomes). The BAC clones used to construct the split probe were obtained from the Human BAC library from the BACPAC resources (Children's Hospital Oakland Research Institute, Oakland, CA, http://bacpac.chori.org). The selected clones flank the TFE3 gene. These BAC clones are: RP11-297F13 (5' TFE3) and RP11-58H17; RP11-184A10 (3' TFE3). BAC DNA was isolated using a Plasmid Midi Kit (Qiagen Inc, Valencia, CA), and labeled with the Vysis Nick Translation Kit. The BAC located at 5' of the gene were labeled using Spectrum Red dUTP, while the BAC located at 3' were labeled with Spectrum Green dUTP. The FISH translocation pattern should be one fusion signal (normal X chromosome) and one red and one green dot (translocated X chromosome) (FISH positive pattern: 1F1R1G). The BAC probes were tested separately against normal metaphases from male control bloods to confirm the probe location. Then, a mixture of selected-labeled BAC probes (TFE3 probe) were tested in normal metaphases and nuclei in order to demonstrate that two fusion signals were clearly seen. TFE3 probe was tested in a patient diagnosed as Xp translocation RCC after cytogenetic analysis of the fresh tumor cells. The karyotype allowed us to detect one metaphase with a t(X;1)(p11.2;p34). Then, we demonstrated a strong nuclear expression of the TFE3 protein in paraffin embedded tissue sections. Before this finding the patient was

underdiagnosed as Clear Cell RCC. FISH was performed on the paraffin-embedded tissue because there was not enough cytogenetic material to apply on metaphase spread. We observed a translocated FISH pattern (1F1R1G) and complex FISH pattern with 1-3 fusion signals and 1-2 split signals indicating a duplication of the translocation. FISH using centromeric X probe revealed cells with three-five signals suggesting an aneuploidy of the X chromosome. We have designed and validated a TFE3 split probe that can be used as a genetic tool in the differential diagnosis of RCC. FISH could be a gold standard technique to detect cases with TFE3 translocation that may be underdiagnosed.

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Technological advances

Phenotypic and genetic characterization of circulating tumor cells by combining immunomagnetic selection and FICTION techniques

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Abstract

Poster presentation

The determination of the number of circulating tumor cells from bone marrow or peripheral blood has been proven to have clinical relevance. However, few studies have analyzed the molecular characteristics of such cells.
We have developed a new method that combines immunomagnetic selection of circulating tumor cells from peripheral blood with FICTION (Fluorescence Immunophenotyping and Interphase Cytogenetics). Peripheral blood (10ml) from healthy individuals was mixed with MCF-7 human breast cancer cells. Tumor-less blood from healthy individuals was used as negative controls. Samples were centrifuged in a double density-gradient to recover tumor cells from the mononuclear and granulocyte cell fractions. Immunomagnetic cell separation using an anti-cytokeratin 7/8 antibody was carried out to isolate tumor cells (CK+ cells). Cells were then placed onto poly-L-Lysine-coated glass slides by cytospin. A primary monoclonal anti-pancytokeratin cocktail antibody [AE1 and AE3], followed by two blue-fluorescent Alexa secondary antibodies [Alexa Fluor 350 rabbit anti-mouse IgG; Alexa Fluor 350 goat anti rabbit IgG] were used to label tumor cells. Then, hybridization with TOP2A/Her-2/CEP17 multicolor probe was used to detect genetic alterations of isolated cells. The cytoplasm of MCF-7 immunoselected cells was specifically labeled with the antibodies, thus showing citokeratin expression. In addition, three centromeric signals belonging to the chromosome 17, two signals for TOP2A, and two signals for Her-2 were detected in the nuclei of the CK+ MCF-7 cells.
Our results demonstrate that tumor circulating cells can be immunoselected, immunophenotyped and molecularly characterized by FICTION analysis. This technique could be useful for the better classification of the patients according to the genetic alterations of the circulating tumor cells, and for the application of targeted-specific therapies.

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Technological advances

Identification of prostate circulating tumor cells by means of the characterization of the TMPRSS2-ERG fusion gene in peripheral blood

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Key words: Prostate cancer, TMPRSS2-ERG, peripheral blood

Abstract

Poster presentation

Introduction: Several chromosomal rearrangements are being identified in prostate cancer (PCa) with already unknown clinical and pathological significance. In at least a 50% of PCa cases the rearrangement consist in a microdeletion at 21q22.3 which leads a fusion gene between the *TMPRSS2*, whose expression is androgen-dependent, and *ERG*, which codifies for a member of the *ETS* family of transcription factors and constitutes one of the partners of other fusion genes in neoplasias of meshenchymal origin. The aim of this study is to employ the detection of the *TMPRSS2-ERG* fusion gene as indicator of circulating tumor cells in patients operated on PCa by radical prostatectomy.
Material and Methods: The study included a total of 30 patients operated between March and July 2007. RNA was extracted from the fixed and paraffin-embedded tissue after the histological examination. From the obtained RNA we proceeded to the *TMPRSS2-ERG* characterization by means of Q-RT-PCR In parallel, a peripheral blood sample was taken before prostatectomy and every 4 month during the follow-up. The sensitivity and specificity of the RT-PCR procedure was studied performing serial dilutions of the VCaP cell line, which express the fusion gene, in the mononucleated cellular fraction of the peripheral blood of a health volunteer (range: 10^6 tumoral cells to 1 cell diluted in 106 normal cells).
Results: The study of sensitivity demonstrated a detection capacity of 10 tumoral cells in one million of normal cells, and no expression of the *TMPRSS2-ERG* was observed in the control of normal cells. The *TMPRSS2-ERG* expression was detected in 17 out of 30 radical prostactectomies (56%), from which in 4 cases (23.5 %) was also detected in the peripheral blood prior to operation. No expression of the fusion gene was observed in any of the blood samples analyzed during the follow-up.
Conclusions: Although the number of cases is still low and the time of followup short, we can say that the detection of *TMPRSS2-ERG* in peripheral blood in those patients whose tumors express the fusion gene is indicative for the presence of circulating tumor cells and could constitute a very useful tool in clinical management of the disease.

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Technological advances

Immunodetection and cytogenetic characterization of disseminated tumor cells applied to the clinical management of patients with solid tumors

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Abstract

Poster presentation

Aim: The aim of the present study was to show that detection and characterization of dissemianted tumor cells in peripheral blood can be applied to the clinical management of patients with solid carcinomas.
Materials and Methods: A double gradient of Ficoll allowed the separation of mononuclear cells, granulocytes and epithelial origin cells from total peripheral blood. Then, for the immunomagnetic selection of the epithelial cells magnetic particles labeled with antibodies against epithelial markers (cytokeratin 7/8 or EpCAM) were used. Finally the disseminated tumor cells were detected using anti-cytokeratin 7/8/18/19 antibodies followed by microscopic visualization. Once detected, cells were cytogenetically characterized by means of In Situ Fluorescent Hybridization (FISH), to detect described chromosomic aberrations for each tumor type using commercially available kits (Vysis®). Five diferent cell lines (A549 lung carcinoma, HT29 colon carcinoma, J82 bladder carcinoma, LNCaP prostate carcinoma and MCF7 breast carcinoma) were used to optimize a method of cell characterization using AMCA immunofluorescence allowing the later visualization of color variety arising from multiFISH hybridizations.
Results: The described methodology detected circulating tumor cells in the blood of 23%, 30% and 43% of prostate cancer (n=21), colorectal cancer liver metastasis (n=94) and bone metastasis (n=16) patients respectively. The number of detected cells ranged from 1 to 16 cells in 6-10ml peripheral blood, with an exceptional case of 100 disseminated cells in a prostate cancer patient. The neoplasic nature of the identified cells was verified through cytogenetic characterization, indicating that both metastatic colorectal carcinoma and bone metastatic breast carcinoma cells show amplification of ZNF217 (20q13) gene. For prostate carcinoma and lung cancer bone metastasis samples ProVysion and LaVysion kits were used respectively. For the detection of aneuploidies in disseminated urothelial bladder carcinoma the UroVysion kit was applied. The fluorescence based detection of disseminated cells made it possible to visualize all the colors of the FISH multiprobe kits, which was impeded when normal immunocytochemistry was used. This method has demonstrated to be applicable to the cytogenetic characterization of the circulating tumor cells and capable of confirming that these cells have the chromosomic alterations that characterize the solid tumos from wich they were released.
Conclusions: The optimized methodology allows the detection and the phenotypical and genotypical charactrization of disseminated tumor cells in routine peripheral blood samples. Its application offers additional information to evaluate clinical prognosis and select the most appropriate treatment, and provides a new tool for the postsurgical monitorization of patients with solid tumors.

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SESSION

Clinical impact of gene changes

&

Future directions

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11th European Workshop on Cytogenetics and Molecular Genetics of Solid Tumours

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Clinical impact of gene changes & Future directions

Novel genomic lesions in patients with unexplained microsatellite instable colorectal tumors

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Abstract

Oral presentation

Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer, HNPCC) is the most common form of hereditary colorectal cancer and is caused by germline mutations in one of the mismatch repair genes MLH1, PMS2, MSH2 and MSH6. Lynch syndrome patients are susceptible for colorectal and endometrial cancer, and to a lesser extent for at least five other carcinomas. Deficiency of mismatch repair in the affected tissues causes microsatellite instability (MSI) represented by insertions or deletions in short repeated DNA sequences. Although MSI can occur in sporadic tumors by hypermethylation of the MLH1 promoter, this extreme manifestation of genomic instability provides an excellent diagnostic marker for hereditary mismatch repair deficiency.

In fact, in a previous study we have shown that in 78% of the families with an MSI-positive tumor and absence of hypermethylation of the MLH1 promoter a disease causing germline mutation in one of the mismatch repair genes could be identified. Nevertheless, in the remaining 22% of the families, the microsatellite instable tumor remains unexplained. In the current study, we set out to identify novel genomic lesions that could affect DNA mismatch repair defects in a cohort of 18 patients with unexplained microsatellite instable tumors using two different genomic profiling strategies. First, we performed high-resolution SNP-based array CGH (Affymetrix) to screen the entire genome for copy number abnormalities. Furthermore, we reasoned that mismatch repair deficiency could also be caused by microRNA gene defects which, considering their size, could have escaped detection with standard high-resolution arrays.

Therefore, we designed a custom-made 385k oligo array (NimbleGen) containing the 695 currently annotated (predicted) microRNAs (NCBI genome build hg18) in 525 clusters on the entire human genome, which allowed us to detect copy number aberrations in microRNA genes smaller than 100 bps in size. We identified several genomic copy number aberrations frequently affecting single genes. One of these lesions directly affects MSH2 expression levels and is present in 40% of the patients with an unexplained MSH2 deficient tumor.

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Clinical impact of gene changes & Future directions

Homozygous deletions may be markers of nearby heterozygous mutations: the complex deletion at FRA16D in the HCT116 colon cancer cell line removes exons of WWOX

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Abstract

Oral presentation

Homozygous deletions in cancer cells have been thought to harbour tumour suppressor genes. We show that this is not necessarily true: we suggest a mechanism that could give a homozygous deletion in a single genetic accident and describe an example where a heterozygous deletion that flanks a homozygous deletion is almost certainly the oncogenic event that is selected. The colon cancer cell line HCT116 has two homozygous deletions of about 25 and 50kb, near each other and within a large intron of the WWOX gene, which spans much of the fragile site FRA16D

We analysed these deletions to the sequence level, using FISH, high-resolution array-CGH and FISH, and found that they result from a complex set of heterozygous deletions, some of which overlap to give homozygous loss. One of the heterozygous deletions has removed exons 6, 7, 8 of one allele of WWOX, resulting in allele-specific expression of a deleted transcript, which is probably the main biological consequence of the deletions, since such deleted transcripts have been found in other cases. A simple model will be presented that shows how such a complex set of deletions could form in a single messy translocation-like event between two homologous chromosomes, so that the selective advantage of such rearrangements need not be within the homozygous deletion.

We conclude (i) that the target of deletions in cancers in the FRA16D region is indeed WWOX, the common outcome being the removal of particular WWOX exons; and (ii) homozygous deletions can be markers of complex rearrangements that have targets outside the homozygous deletion itself.

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Clinical impact of gene changes & Future directions

Is the DNA damage response a cancer barrier?

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Abstract

Oral presentation

Is the DNA damage response (DDR) a bona fide tumor suppressor barrier? Recent data on oncogene-induced senescence and the finding of an active DDR in early stages of cancer would suggest so. In particular, the model postulates that oncogene-induced DDR activation is linked to the generation of "replicative stress". In this scenario, oncogenic mutations should mainly impinge on ATR (rather than ATM) activation. However, understanding the physiological functions of the ATR/Chk1 pathway has been hampered by the early embryonic lethality of knockout mice. Moreover, no fully deleterious mutations of the pathway have ever been described in tumous or cancer cell lines, which further underscores the essential nature of this pathway. We have now developed a couple of genetic tricks to circumvent these problems and generated mouse models in which we hopefully would be able to address the role of ATR in the context of a mammalian organism. The models, our initial findings and plans for their use will be discussed.

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Clinical impact of gene changes & Future directions

Prognostic implication of TMPRSS2-ERG fusion gene in patients with prostate cancer operated by radical prostatectomy

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Key words: Prostate cancer, TMPRSS2-ETS, prognosis

Abstract

Poster presentation

Introduction: To date any of the described molecular alterations in prostate cancer (PCa) has been clearly specific for the tumor. Recently, different fusion genes have been identified between *TMPRSS2* (21q22.3) with some of the members of the ETS family of transcription factors [*ERG* (21q22.3), *ETV1* (7p21.3) or *ETV4* (17q21)] which specifically are involved in at least 40-50% of PCa. The aim of this study is to determine the prognostic significance of the expression of the *TMPRSS2-ETS* fusion gene in a retrospective series of PCa.
Material and Methods: One hundred and twenty-three fixed and paraffin-embedded tissues from radical prostatectomies comprising the period 1996-2000 (median of follow-up: 95.86 months, range: 0.17-139.03) were analyzed. Sixty-one out of 123 patients (49.6%) progressed biochemically during the follow-up. After histopathological examination RNA was extracted and the expression of *TMPRSS2, ETV4, ETV1* and *ERG* was evaluated by means of quantitative RT-PCR The presence of the *TMPRSS2-ERG* fusion transcript was also evaluated using the VCaP cell line, which expresses the fusion gene, as positive control. Univariate (log rank) and multivariate (Cox regression) analysis were performed to determine the prognostic implication of the clinical, pathological and molecular variables for the biochemical progression (high serum PSA levels).
Results: The expression of *TMPRSS2-ERG* was observed in the 44.7 % of PCa and no correlation with any of the clinical and pathological parameters was observed. Only the expression of *ETV4* was inversely correlated with initial serum PSA levels (p=0.049). For the whole series serum PSA levels (p=0.0003), stage (p=0.0194), extraprostatic disease (p=0.0004), Gleason (p=0.012) and lymph-node involvement (p=0.0068) had a prognostic significance for the biochemical progression, the PSA level, the Gleason score and the extraprostatic disease having an independent prognostic value after the multivariate analysis. When we analyzed the cases separately according to the *TMPRSS2-ERG* expression status we observed a different behaviour as is showed in the following table:

No TMPRSS2-ERG expression			TMPRSS2-ERG expression		
Parameter	**%BPFS**	**P**	**Parameter**	**%BPFS**	**p**
PSA levels	66 vs 35 vs 33	0.0234	PSA levels	56 vs 70 vs 9	<0.0001
Stage	56 vs 0	0.0023	Gleason score	60 vs 25 vs 0	<0.0001
Extraprostatic disease	70 vs 30	0.0003			
Lymph-node involvement	53 vs 25	0.0317			

(BPFS, Biochemical progression free survival)

After the multivariate analysis we observed that in the group of PCa which no expressed TMPRSS2-ERG, the stage [HR=4.8 (1.9-12)], the extraprostatic disease [HR=4.1 (1.9-8.9)] and the lymph-node involvement [HR=2 (2-26)] had an independent prognostic value. However, for the group of PCa with expression of *TMPRSS2-ERG* only the PSA levels [HR= 5.6 (1.8-11.2)] and the Gleason score [HR=15 (0.9-250)] showed an independent prognostic value.

Conclusions: Although these results are still provisional and a larger series of cases are being analyzed we can conclude that *TMPRSS2-ERG* expression defines a group of PCa with a different behaviour and these findings could change the clinical management of PCa patients.

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