Conclusions: The present study suggested that cancer patients with TE should be evaluated for FVL but PT G20210A was not contributing factor to be development of TE during cancer treatment.

254 PUBLICATION

The RBBP6 expression in oesophageal tumours

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The RbBP6 is a 36 kb gene and containing 18 exons. It is transcribed to three mRNA transcripts, 1.1 kb and 6.1 kb that have a splice variant missing exon 16 splice. Deletion or mutation of the 1.1 mRNA variant in CHO cells have been found to render cells resistant to apoptosis induced by chemical inducers such as staurosporine and this directly links RbBp6 gene to apoptosis.. The l.1 mRNA is translated into a 13 kDa protein (isoform 1) containing the DWNN domain only whereas the 6.1 mRNA is translated to 200 kDa proteins isoform 2 and 3 having the RING Finger, Rb and p53 binding domains linked to the DWNN domain.
The aim of the study was to determine the expression pattern and tissue distribution of RbBp6 gene products in oesophageal tumours. We have studied poorly, well and moderately differentiated human squamous oesohageal tumours. We have also compared the levels of expression and apoptosis in these tissues.
Using both in situ hybridization and immunocytochemistry, we have found that RbBP6, is found upregulated and we also found that it accumulates in the cytoplasm like the mutated p53. In normal tissues RbBp6 was found to be localizing mostly in nuclei and rarely in the cytoplasm. We have found that RbBp6 is upregulated around islands of tumours in well differentiated squamous tumours where the apoptosis is high and very much involved in fighting the invading tumours and found none or little RbBP6 localization in the islands of tumours where apoptosis had completely halted. The RbBP6 expression level correlated with apoptosis and was found to be inversely proportional to proliferation as it was shown by TUNEL and Ki67 respectively. We have also used real time quantitative RT-PCR using Roche LightCycler and have confirmed that the RbBP6 expression levels are increased in oesophageal tumours as compared to normal oesophageal tissues.
The RbBP6 200 cDNA had previously been cloned by detecting its interaction with tumour suppressor proteins p53 and Rb, which have a major role in apoptosis and cancer development. Accumulation of these proteins, p53 and RbBP6, suggests that RbBP6 may be involved in a p53 dependant apoptotic pathway.

255 PUBLICATION

Prediction of the response to radiotherapy by comet assay: preliminary results in cervical tumors

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Background: Intrinsic cellular radiosensitivity (RS) is a genetic factor involved in the interindividual variability of the response to radiotherapy (RT). A major research goal is the establishment of a predictive assay based on individual radiobiologic characteristics. Comet Assay might be a reliable assay to be used in the prediction of clinical RS.
Purpose: The specific aim of our prospective study is to correlate the *in vitro* RS of tumor cells with the clinical response after curative RT.
Material and method: Twelve patients with locoregionally advanced cervical carcinoma were included so far. Tumor cells obtained from short time primary cultures of tumor tissue prelevated by biopsy were irradiated *in vitro* and analysed by Comet Assay. For each tumor two parameters of the degree of DNA lesions (Lesion Score-LS and Tail Factor-TF) were scored before, immediately after and at two hours after irradiation. Tumor response was clinically assessed at the completion of the treatment as follows: stationary disease (SD), partial response (PR) and complete response (CR). *In vitro* parameters of RS were correlated with the clinical results.
Results: According to the degree of DNA lesions, 3 biological parameters were evaluated: the background level (B), the magnitude of induction by irradiation (I), and the repair of the radioinduced lesions (R). Differences in B reflect the interindividual variability of the sensitivity to ionizing radiations. Treatment results (clinical responses at the end of RT) correlated with I and R. All 5 cases with CR were characterized by high or moderate I and /or defficient R; 2 cases with good PR had low I and lack of R. The 5 cases with SD showed low I and good R.
Conclusions: Comet assay is a modern, quick and reproducible method which seems to be a promising tool for the prediction of the clinical response to RT. Our preliminary results are encouraging but a larger number of patients must be included in order to draw reliable conclusions.

256 PUBLICATION

Flowcytometric accurate determination of ABC-transporters' activity regulating anthracycline intracellular compartmentalization in multidrug resistance cells

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Background: Intracellular compartmentalization is the major determinant of classical multidrug resistant mechanism (MDR) associated with ABC-transporters' function activity. The purpose of the study is to develop an accurate approach for separate determination of of ABC-transporters' activity regulating nuclear and cytoplasmic accumulation of MDR-drugs.
Material and methods: ABC-transporters' functional activity (MDR-phenotype) was determined as the change in doxorubicin (Dox) intracellular accumulation (ICA) under action of specific inhibitors of Pgp and MRP by flowcytometry. The following new data are the result of more than 100 biopsy sample investigation (breast, colon and cervix carcinoma).
Results:
1. Analyzing the results of MDR-phenotype study it has been revealed an interesting phenomenon: increase in Dox ICA under inhibitor action is accompanied by two opposite changes in Dox intracellular fluorescence (ICF): increase or decrease of the index.
2. Under the same inhibitor concentration the change in Dox ICF depends on tumor cell investigated.
3. The direction of the change in Dox ICF in the same cells depends on inhibitor concentration: increase in Dox ICA is accompanied by decrease in Dox ICF under action of higher inhibitor concentration but index increases in lower inhibitor concentration.

Conclusions: 1. Well-known phenomenon of Dox fluorescence quenching as a result of anthracycline binding to DNA let us conclude that decrease in Dox ICF means main increase in nuclear Dox accumulation and binding to DNA under inhibition of ABC-transporters regulating Dox accumulation in the nucleus. On the contrary, increase in Dox ICF results from main increase in cytoplasmic Dox accumulation under inhibition of ABC-transporters regulating cytoplasmic Dox accumulation. 2. Nuclear ABC-transporters are more resistant to inhibitors' action. 3. So, investigation of Dox ICF changes under ABC-transporters' inhibition by flowcytometry make it possible determination of anthracycline intracellular distribution and separate estimation activity of ABC-transporters regulating nuclear and cytoplasmic accumulation of the drug. The latter is the most important index of MDR-phenotype for prognosis of resistance to chemotherapy in cancer patients.
Supported by Russian Foundation for Basic Research (04–04–49391).

Breast Cancer

Oral presentations (Thu, 3 Nov, 8.30–10.35)

Molecular characterization of breast cancer and its clinical implications

257 ORAL

Combination of two biological gene expression signatures in predicting outcome in breast cancer as an alternative for supervised classification

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Introduction: Gene expression profiling has been used to identify specific subgroups of breast carcinomas that differ with respect to clinical and pathological features, including outcome. We have previously identified 3

different classifiers (70 genes "prognosis profile" (supervised on clinical outcome), Wound Signature (WS) and Hypoxia Signature (HS), both unsupervised) that separate patients into relatively good and poor prognosis groups. The supervised approach has an excellent sensitivity, but somewhat lower specificity for metastasis free survival. The unsupervised approaches have a higher specificity to identify patients with poor prognosis, but a relatively low sensitivity. In order to optimize both sensitivity and specificity, we combined the unsupervised profiles.
Methods: In a previously described series of 295 stage I and II breast carcinomas treated at the Netherlands Cancer Institute, we have obtained gene expression data of 25,000 genes using micro-array analysis. We have categorized the patients according to previously established groups. The first group consists of patient with a quiescent WS and a non hypoxic signature, patients in the second group have either an activated WS or hypoxic signature and the third group consists of patients with both an activated WS and a hypoxic signature.
Results: At a median follow up of 12 years for patients alive, the metastasis free probability (MFP) at 12 years is 79% for group 1 (n = 110), compared to 64% for group 2 (n = 103) and 45% for group 3 (n = 82) (log rank: p < 1*10−6. HR: 2.1 (95%CI: 1.6−2.7)); these figures were 87%, 68% and 37%, respectively, for overall survival (OS) (p < 1*10−12. HR: 2.6 (95%CI: 2−3.4). In subgroups with a favorable clinico-pathological characteristics (pT1N0, ER+ and pN0) the predictive power remains highly significant, as in patients with unfavorable clinico-pathological characteristics (pT2N+, ER− and pN+). The true negative predictive value for OS for group 1 is 87% and the true positive predictive value for group 3 is 60%. In multivariate analysis the combining the WS and HS signatures resulted in the best prediction of MFP and OS, which was independent of clinico-pathological variables (ER, TN-stage, Grade, Angioinvasion, Chemotherapy and age).
Discussion: In this consecutively treated series of breast cancer patients, the combination of the Wound Signature and Hypoxia classification stratifies patients that differ with respect to prognosis in three risk categories. Combining different gene expression signatures may result in improved classification of breast carcinomas.

258 ORAL

Utilisation of microarray technology to refine molecular classes and improve clinical management of breast cancer

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Background: The significant genetic heterogeneity among breast cancer patients is a primary obstacle to effective clinical diagnosis and management. Emerging technologies based on gene expression profiling (GEP) may provide clinically useful information to improve the management of breast cancer. GEP has been used to refine classification of previously undistinguishable tumour subgroups, and predict prognosis and response to anticancer agents. Here we report a multicentric GEP analysis to identify and validate predictors in order to improve tumour classification and predict patients most likely to respond to standard chemotherapy.
Material and methods: 323 patients with early breast cancer treated with adjuvant anthracycline-based chemotherapy were selected from Institut Paoli-Calmettes (IPC), Marseille and Centre Léon Bérard (CLB), Lyon. RNAs were analysed on 10 K nylon cDNA microarrays. Metagenes for tumour classification were identified based on adjusted t-test analysis and hierarchical clustering on an identification set. A Cox-based method was used to find predictors able to discriminate patients with favourable outcome (no metastasis) after chemotherapy, by combining validated metagenes with clinical factors on an identification set of 159 patients treated with anthracyclines (IPCa). The stability and robustness of these predictors were assessed on two different and independent validation sets (IPCb n = 54 & CLB n = 110). The best predictor was compared with the Nottingham Prognostic Index (NPI).
Results: A predictor was identified on patients treated with chemotherapy (anthracyclines). This predictor was based on a linear combination involving metagenes and clinical factors, i.e. A*(metagene 1)+B*(metagene 2)+C*(clinical factors). It classified patients in two groups with different outcome. The robustness of this predictor was then confirmed on the two validation sets of patients. Our predictor compared favourably with the NPI, improving the classification of the low-risk patients.
Conclusions: Our metagene-based predictor is highly efficient to discriminate patients with favourable outcome under adjuvant anthracycline-based chemotherapy. It uses a validated combination of genes known for their biological relevance, and is valid irrespective of the clinical centre. Additional clinical studies and technical developments are ongoing to translate this new tool into a decentralised test designed for routine clinical practice.

259 ORAL

Standardisation of HER2 testing: results of an international proficiency testing ring study

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Background: HER2-positive breast cancer indicates aggressive tumour growth, poor prognosis and treatment response to trastuzumab. Early and accurate determination of HER2 status is essential for optimal management of breast cancer. Because current HER2 tests (immunohistochemistry [IHC], fluorescence in-situ hybridisation [FISH], and chromogenic in-situ hybridisation [CISH]) are reader-dependent, validation by laboratory proficiency testing is important to improve standardisation. The study compared IHC and FISH testing between five international pathology reference centres.
Methods: A total of 20 IHC and 20 FISH breast cancer specimens were evaluated separately in five testing rounds (8-week intervals). In each round, a designated laboratory selected two sets of four different invasive tumour specimens (set A for IHC [HercepTest™]; set B for FISH [PathVysion]). The lab sent samples from each set to the other four testing centres in a blinded fashion, while retaining samples for its own evaluation. IHC scores were stated as negative (0, 1+), ambiguous (equivocal, 2+) or positive (3+). FISH scores were based on the ratio of HER2 signals to chromosome 17 centromere signals: negative (<2.0) or positive (⩾2.0). At the end of each round, an independent co-ordinator analyzed and discussed the results among the centres.
Results: All centres reported the same findings for nine out of 20 IHC specimens (45%). Although reports differed in the remaining 11 specimens, there were no cases wherein a laboratory reported a specimen as HER2 positive and another reported it as negative. At least one laboratory reported an ambiguous HER2 status in each of the 11 specimens. Sixteen out of 20 (80%) FISH specimens had similar scores from all the centres. The four cases in which the centres did not agree had mean amplification levels of 1.95, 1.48, 1.72 and 1.82. In the second of these cases, the difference in the report was due to one centre reporting a value of 2.0, while the others reported <2.0.
Conclusions: Equivocal IHC and borderline FISH cases are difficult to interpret, even for highly experienced and validated laboratories. To help determine the treatment, FISH retesting of IHC 2+ samples and retesting of FISH borderline cases with FISH, IHC, or CISH is recommended. As a follow-up of this study, equivocal IHC samples will be retested by FISH. Each testing laboratory should regularly validate their HER2 testing to ensure proper reporting of test results.

260 ORAL

Comparison of her2/neu expression on the primary tumor and on isolated tumor cells in the bone marrow of breast cancer patients

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Background: There is growing evidence that the presence of isolated tumor cells in the bone marrow (ITC) of breast cancer patients, both at primary diagnosis and during follow-up, indicates an increased risk for subsequent recurrence (Braun, NEJM 2000; Janni, Cancer 2005). Therefore, ITC might be a potential target for tailored treatment options in these patients. Aim of this study was to establish a new method to analyse cytokeratin-positive (CK+) cells for her2/neu gene amplification.
Methods: ITC were detected using a standardized immunoassay with monoclonal antibody A45-B/B3, directed against cytokeratin 8, 18, 19 (CK) and stained according to the APAAP-technique. 2×10^6 cells per patient were screened by bright field microscopy. Subsequently, cytospins with CK-positive cells were further characterized by fluorescence in situ hybridisation (FISH) using a her2/neu DNA probe (Zymed, Germany) or a multi-colour probe (Vysis, Il., USA) for hybridisation of centromer 17 (polyploidy) and the her2/neu growth factor gene. A ratio of her2/neu and centromer chr. 17 signals of at least two was regarded as amplification.
Results: 232 bone marrow aspirates of 156 patients with breast cancer were analyzed at the time of primary diagnosis and during follow-up. ITC were detected in 68 samples (29%) in this patient group. The median number of detected cells was 2 (range 1−58). In 45 randomly assigned aspirates with ITC, the her2/neu status on these cells was evaluated and