patients with BRCA1-deficiency. The ability to identify these patients by gene expression profiling from FFPE derived breast tissue may also have significant clinical application.

631 POSTER

Mislocalization of the apoptosome protein Apaf-1 is a strong marker of drug resistance in B cell lymphomas

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Chemotherapy resistance remains a challenge in the clinical management of diffuse B-cell lymphomas despite aggressive treatment with CHOP and monoclonal CD20. We previously reported that sequestration of Apaf-1 to membrane lipid rafts was responsible for apoptosis resistance in B-cell lymphoma cell lines [1]. Here, we extended our studies to clinical biopsies from patients with B-cell lymphomas, T-cell lymphomas and reactive lymphadenopathy, to investigate if the resistance to drug-induced apoptosis was, indeed, a function of Apaf-1 mislocalization. Firstly, cells were separated from these biopsies and their sensitivity to a variety of apoptosis inducing agents was assessed. Whereas most T-cell lymphomas as well as reactive lymphadenopathy cells were sensitive to apoptotic stimuli, B-cell lymphomas exhibited strong resistance. We then investigated the expression of Apaf-1 and its intracellular localization in these clinical biopsies. To do so, cell fraction was performed to separate cytosol from membrane-enriched fractions. The latter were further subjected to density gradient centrifugation to obtain lipid raft fractions. We show that Apaf-1 was expressed in total cell lysates from B- and T-cell lymphomas, however upon fractionation the localization was strikingly different. In T-cell lymphoma samples as well as in cells derived from reactive lymphadenopathy biopsies, Apaf-1 expression was prominently detected in the cytosol, which correlated with the sensitivity of the cells to apoptotic stimuli. In contrast, whereas cytosolic Apaf-1 expression was significantly lower or absent in almost all B-cell lymphomas analyzed, increased localization of the protein was detected in membrane lipid rafts. The latter was confirmed by immunohistochemical analysis of tissues from the same biopsy specimens. Interestingly, the resistance of B-cell lymphomas to apoptotic execution (drug-induced or death receptor-mediated) was significantly bypassed upon incubation of cells with pharmacological agents that facilitated the dissociation of Apaf-1 from the lipid rafts to the cytosol. Taken together, our results implicate Apaf-1 mislocalization as a potential diagnostic marker for B-cell lymphomas as well as a predictor of response to therapeutic management. This work is supported by research grants to S.P. from the NMRC, Singapore, and the Singapore Cancer Syndicate.

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632 POSTER

Expression of a+β+ splice variant of human telomerase reverse transcriptase (hTERT) in cytokeratin 19 (CK-19) positive circulating tumor cells (CTCs) of breast cancer patients

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Introduction: Human telomerase reverse transcriptase (hTERT) is the catalytic subunit of telomerase. The α+β+ splice variant is the functional variant of hTERT. Circulating tumor cells (CTCs) have already been established as strong predictors of prognosis in patients with metastatic breast cancer. Our group has previously shown the prognostic significance of cytokeratin 19 (CK-19) mRNA-positive CTCs in early breast cancer. The aim of our study was to study the expression of hTERT α+β+ splice variant in CK-19 positive CTCs in breast cancer samples by qRT-PCR.
Materials and Methods: Peripheral blood (20 ml in EDTA) was obtained from 25 patients with early breast cancer before the administration of adjuvant chemotherapy, 14 patients with verified metastasis who were all tested positive for CK-19 expression by real time PCR (Stathopoulou et al, Int J Cancer 2006), and 17 female healthy volunteers. CTCs were isolated after ficoll density gradient centrifugation, following enrichment with immunomagnetic Ber-EP4 coated capture beads, mRNA was isolated using oligo $(dT)_{25}$ coated magnetic beads, followed by cDNA synthesis. The expression of hTERT α+β+ splice variant was tested in both CTCs and PBMCs fractions by quantitative real-time PCR in the LightCycler, (Mavroyiannou et al, Clin Chem, 2007).
Results: hTERT α+β+ splice variant was expressed in 4/14 (29%) of CK-19 mRNA positive CTCs samples from patients with metastasis and in 5/24 (21%) of CK-19 mRNA positive CTCs samples from patients with early breast cancer. None of the 17 female healthy volunteers CTCs fraction was tested positive for hTERT α+β+ splice variant, while the corresponding PBMCs fraction was positive in all cases.
Conclusions: To our knowledge this is the first report on the expression of hTERT α+β+ splice variant in CTCs of patients with early breast cancer and verified metastasis. Further studies are needed to evaluate and confirm our findings in a larger number of patients.

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MicroRNA-21 expression levels are accompanied by respective alterations in PDCD4 protein levels in non-small cell lung cancer

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Background: The aim of our study was to investigate the correlation between mature miR-21 expression levels and programmed cell death 4 (PDCD4) protein levels in non-small cell lung cancer.
Materials and Methods: Forty pairs of NSCLC fresh-frozen tissues and their corresponding noncancerous tissues were analyzed for the expression of mature miR-21 using quantitative real-time RT-PCR, as previously described (Markou et al., 2008). In parallel, PDCD4 protein levels were evaluated by immunohistochemistry. Deparaffinized sections cut from paraffin-embedded tissue samples were stained with a specific anti-PDCD4 antibody (1:100 dilution) (Ozaki et al., 2006) using HRP DAB kit (DAKO) for the detection. The tumor types and stages were determined according to the WHO classification. All samples were analyzed histologically to access the amount of tumor component (at least 70% of tumor cells) and the quality of material.
Results: Among the 40 NSCLC tissue specimens studied, suppresion of miR-21, in respect to their adjacent non-neoplastic tissues, was detected in 24 samples (60 %). In 15 out of these 24 patients (62.5%), we observed that the supression of miR-21 was accompanied by increase of PDCD4 protein levels. Mature miR-21 was overexpressed in 16 out of 40 patients (40%), and in 8 out of these 16 patients (50%) we detected reduced PDCD4 protein levels. Totally, in 23 out of 40 samples (57.5%), the altered miR-21 expression levels correlated with changed PDCD4 protein levels.
Conclusion: Our data indicate for the first time that PDCD4 protein expression levels are regulated by miR-21 in non-small cell lung cancer tissues.

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Patient-derived breast cancer xenografts: Molecular characteristics and growth properties

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Breast cancer is a heterogeneous disease for which several subtypes have been described according to histological and molecular determinants. Therapeutic decisions are dependent on tumor staging which includes molecular markers like estrogen receptor (treatment with anti-estrogens or aromatase inhibitors) or HER2 (treatment with HER2 targeting agents Trastuzumab or Lapatinib).
Here we present a panel of 12 patient-derived breast cancer xenografts, eleven of which have been established by Oncotest from primary patient material. Molecular profiling included expression analysis of receptors for estrogens (ER), androgens (AR) and progesterone (PR), HER2 and the E2F transcription factor 1 by quantitative RT-PCR and Affymetrix HG U133 plus2.0 array analysis. HER2 protein and phosphorylation were determined by ELISA and − for selected models − FISH analysis was performed to determine gene amplification. MAXF 713 and MAXF 1398 are ER positive, luminal B subtype tumors, whereas MAXF 1162 is a HER2-overexpressing, Lapatinib sensitive tumor. The high HER2 levels in MAXF 1162 are based on a gene amplification. MAXF 1322 is borderline HER2-positive, but insensitive towards Lapatinib and Herceptin. The majority of tumor models (8 out of 12) belong to the basal-like, triple negative breast cancer subtype.