and Cox proportional hazard models showed that miR-221 down-regulation was linked to tumor progression and recurrence.
Conclusion: Our results suggest that progressive miR1-221 down-regulation is a hallmark of metastasis and a novel prognostic marker in prostate carcinoma. This suggests that miR-221 has potential as a diagnostic marker and therapeutic target.

PP59

Prognostic significance of αB-crystallin, vimentin and HSP 27 association in primary breast cancer

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Background: αB-crystallin is a heat shock protein, which function as stress-induced molecular chaperones to inhibit the aggregation of denatured proteins. Previous studies have identified αB-crystallin as a marker of poor prognosis for breast cancer and have suggested that it is an excellent marker for tumours of basal origin. We have considered that αB-crystallin binding proteins, vimentin and HSP27 also show a similar association with poor prognosis.
Materials and Methods: Tissue Micro Arrays of 0.6 mm cores of 246 breast cancers were stained with antibodies to αB-crystallin, vimentin, HSP27 (antibody ERD5) and HSP27 82P and scored using the Quick Score Method. The results stored with the Aperio Pathology Database were then subsequently compared with clinical and pathological parameters.
Results: Expression of αB-crystallin was associated with vimentin [$P < 0.001$ Fishers exact test (FET)]. αB-crystallin expression was linked to a low expression of the estrogen receptor and reduced survival ($P < 0.001$ (FET), $P = 0.002$ Kaplan Meier Log Rank (KM) respectively). Vimentin expression was associated with estrogen receptor (ER) negative cancers and poor survival ($P < 0.001$ (FET), $P = 0.002$ (KM Log Rank) respectively). In contrast to αB-crystallin, low expression of HSP27 was associated with low ER and progesterone receptor (PGR).
Conclusion: Increased expression of the protein chaperon, αB-crystallin and its binding partner, vimentin were linked to reduced survival. A similar association was not found for HSP27 expression. The potential functional significance of this interaction for metastasis will be discussed in the context of other predictive markers for breast cancer.

PP118

Detection and quantification of EGF receptor phosphorylation in formalin-fixed tumor sections by selected/multiple reaction monitoring mass spectrometry

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Background: The epidermal growth factor receptor (EGFR) is a drug target in several cancers, but suffers from a lack of molecular biomarkers to facilitate the selection, monitoring and dosing of patients. Mass spectrometry (MS) has emerged as a sensitive method to track not only the EGFR, but to monitor specifically sites of phosphotyrosine (pY) on the EGFR and the protein components of its signaling network that may serve as biomarkers of EGFR expression and activity. Major challenges in the development and application of MS as a means to discover and assay biomarkers, and in particular phosphorylation-type protein features related to drug target modulation, include (i) the preservation of protein-phosphorylation in patient samples, and (ii) the detection and quantification of such features in minute, heterogeneous patient samples. To address these challenges we have combined Liquid Tissue technology, which enables solubilization of protein from cells obtained by laser microdissection of formalin fixed patient samples, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation.
Materials and Methods: Liquid Tissue technology was used to solubilize protein from formalin fixed tissue samples. Solubilized, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation. This approach was applied to measure features of the EGFR network in formalin fixed tissue culture cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples.
Results: EGFR peptides were measured by direct SRM/MRM analysis of trypsin-digested, liquefied samples from formalin fixed cultured cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples. Enrichment of phosphorylated peptides by using titanium dioxide resins enabled the measurement of EGFR phosphorylation sites reflecting activated EGFR.
Conclusion: These results provide proof of concept for a robust approach to monitor in tumors the EGFR and other phosphorylation-associated drug targets and biomarkers, and which may offer superior dynamic range and quantification over traditional immunohistochemistry-based methods.

PP94

The Chernobyl Tissue Bank − a model for integrating "omics" research on single blocks of tissue

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Background: The Chernobyl Tissue Bank (CTB) was established in 1998 to collect, store and distribute biological samples from patients resident in the regions of Ukraine and Russia contaminated by fallout from the Chernobyl accident and who developed thyroid cancer. Patients give generic (broad) informed consent for thyroid cancer research; access to biomaterials is approved by an external review panel. A sample of blood for extraction of DNA, serum and samples of both frozen and formalin fixed paraffin embedded (FFPE) tumour and normal thyroid tissue are provided from each patient.
Materials and Methods: The current collection includes 2493 cases of thyroid cancer and adenoma. RNA and DNA are extracted from the same frozen tissue block and are distributed to researchers in aliquots of 5 μg (RNA) and 3 μg (DNA), permitting multiple projects to have access to material from the same block of tissue. A frozen section is taken from each block prior to extraction and the relative proportions of epithelial, stromal, lymphoid cells are assessed. Quality assurance (QA) is carried out by Aglient Bioanlyser (RNA–RIN) and 10 kb gel (DNA), enabling samples only of the highest quality to be provided to projects that require this e.g. Affymetrix 3′ array.
Results: A recent QA audit showed that the average RIN was 8.5 (range 6.4−9.4). There was no significant degradation over a 10 year period of storage as a frozen block prior to extraction. 1631 aliquots of RNA and 703 of DNA from tissue, 136 aliquots of DNA from blood and 5921 sections from FFPE blocks have been issued to researchers worldwide. The research projects supported by the CTB range from single gene investigations to complex projects using a variety of array based platforms. One example is Genrisk-T, an EC funded project is currently combining mRNA array, bac array (on RNA and DNA extracted from a single frozen tissue block) and germline SNP data with miRNA (from FFPE material of the same case) and clinicopathological data on an age-matched series of 50 patients who were exposed to radiation and 50 who were born after 1/1/87 and have developed spontaneous thyroid cancer at a young age. The aim of the study is to identify radiation related changes and novel genes in thyroid cancer.
Conclusion: The CTB is being used by others (e.g. the Wales Cancer Bank) and by clinical trial groups as a paradigm for a tissue bank to support integrated "omics" research on other tumour types.

PP85

Genotyping of microsatellite alterations and EGFR somatic mutations in exhaled breath condensate of NSCLC patients

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Background: A common goal in treatment of NSCLC patients is to individualize genetic and epigenetic events which can be used as early diagnostic marker and which could be easily and time-saving investigated. We recently demonstrated the possibility of studying microsatellite alterations (MAs) in the DNA of exhaled breath condensate (EBC). The aim of the present study was to verify whether MA analyzed in DNA from EBC can be used to detect tumor susceptibility in high risk subjects studying microsatellite alterations and whether it can be useful to detect EGFR more common mutations in lung cancer.
Materials and Methods: 59 subjects entered the study: 41 with NSCLC and 18 with non-neoplastic diseases. All subjects underwent allelotyping on DNA from whole blood, EBC, and lung tissue removed for histologic diagnosis by analyzing a panel of five microsatellites (D3S2338, D3S1266, D3S1300, D3S1304, D3S1289) located in chromosomal region 3p. Among the overall series, 23 patients were also investigated for EGFR mutations in exons 18−21 on DNA from EBC and paraffin embedded tumor tissues.
Results: MAs in DNA from tumor tissues and EBC of each patient with cancer presented an overlapping profile of loss of heterozygosity (26%)