factors for BC could partly explain the gene expression differences in the cases. In order to increase the repertoire of informative genes we are extending the analyses to a population based prospective study (NOWAC, Dumeaux et al 2008, BCR) with a larger number of BC and non-BC samples performing large-scale gene expression analysis. In this cohort changes in blood-derived gene expression profiles were observed for HRT users compared to non HRT users (Dumeaux et al 2006 Mol Cancer Ther). Thus, gene expression changes as a diagnostic test for BC may have to be adjusted for confounding factors related to different exposures (e.g. hormone exposure).

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Gene methylation for the diagnosis and prognosis of cancer

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Alterations in DNA methylation, an epigenetic process present in mammalian cells, are a hallmark of human cancer. Aberrant hypermethylation of the promoter region of genes is associated with loss of function of the gene. A number of genes, many implicated in important biological pathways, are known to be methylated in cancer. Gene hypermethylation can occur frequently and early in the development of cancer. Sensitive methylation specific PCR technology exists that permits detection of gene methylation in tumor cells in tissue biopsies, urine, blood and other body fluids. Conceptually, tumor suppressor gene methylation is highly specific for neoplastic cells. For these reasons, methylation is a promising target for the detection and prognosis of cancer in tissue biopsies and body fluids.

Gene methylation has been identified by examination of individual candidate genes and, more recently, by global screens such as demethylating drug-based reactivation of expression in tumor cell lines. Bisulfite sequencing and MSP technology are used to assess methylation status. The effect of promoter methylation upon transcription is examined by Northern, RT-PCR, Western or immunohistochemical analysis. Studies of the frequency, timing and neoplastic cell specificity of individual genes by sequencing or MSP analysis have identified genes suitable as targets for early detection of cancer. The pattern of genes methylated in particular tumors has been studied for predictive power for stratification of a cancer patient's outcome or response to a certain therapy

Feasibility studies have demonstrated sensitive and specific detection of gene methylation in tissue biopsies and non-invasive body fluids from patients with cancer of an early stage when treatment can result in a better outcome. Global screens are leading to the elucidation of the cancer cell methylome. The average total number of genes methylated with functional significance in a tumor cell is unknown but might be reasonably estimated as several hundred. Mining of this data can improve current panels of genes used for early detection studies and extend such panels to provide signatures for differential diagnosis and prognosis. Correlative studies of gene methylation with clinicopathological parameters have highlighted potential markers of tumor behavior including response to therapy.

Challenges for methylation-based detection include: the likely need for larger panels of methylated genes in detection, optimization and standardization of specimen processing and technology for analysis, further study of gene methylation in normal or non-neoplastic cells, knowledge of timing of methylation of a gene in regard to clinically significant disease, and the ability for differential diagnosis of the anatomical site of origin of a tumor in a body fluid. Elucidation of the cancer cell methylome is accelerating and providing new candidate methylated genes for early detection and predictive classification of tumor behavior. In addition, new technologies are emerging that may allow more comprehensive and robust study of aberrant gene methylation in cancer cells.

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Predictive gene profiles for breast cancer patients

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The use of gene expression assays in clinical medicine is a goal that will only be realized after validation of the technology and potential classification lists. Our previous gene expression profiling studies identified five major molecular intrinsic subtypes of breast cancer (Luminal A, Luminal B, Basal-like, HER2+/ER-, Normal Breast-like) that show significant differences in patient survival and metastatic potential. Mounting evidence also suggests that these subtypes vary in their responsiveness to chemotherapeutics and biologic agents. In fact, the expression of drug targets like HER1 and HER2, within selected subtypes only suggests that logical combinations of chemotherapeutics and biologic agents will be subtype-specific; however, this must be empirically evaluated. Nonetheless, selecting the right chemotherapeutic(s) and biologic agent combination for each subtype has yet to be experimentally or clinically determined. Therefore, I will present the available data concerning the responsiveness of the intrinsic subtypes relative to neoadjuvant chemotherapy and tamoxifen.

In addition to the prognostic and predictive abilities of the intrinsic subtype classification, a number of other groups have also identified gene sets and methods that predict outcomes in breast cancer patients including the 70-gene profile of van't Veer et al. (MammaPrint™), the Genomic Health Recurrence Score Assay (Oncotype DX™). Using a single data set of 295 patient/samples from the Netherlands Cancer Institute, each sample was assigned a class for each of these predictors. Within the Basal-like, Luminal B and HER2+/ER- tumor subtypes, great concordance across all predictors was observed. However, within the Luminal A group, there was heterogeneity in predictions, suggesting that further stratification with this largest group is still needed and could be provided by the MammaPrint or Oncotype DX assays. These data will be presented in more detail including a discussion of additional gene expression predictors and how these data can be used to guide current therapeutic decision making.

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TP53 as an important molecular marker

No abstract received.

05 July 2008 17:00 - 18:00

PLENARY LECTURE

Cancer stem cells

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Breast cancer stem cells

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Stem cells are defined as cells with self-renewal capacity and the ability to give rise to multiple differentiated cell types. Recent in vitro and in vivo clonality and tumorigenicity studies have demonstrated the existence of cells with stem cell like properties both in normal human breast tissue and breast carcinomas that are required for normal mammary gland development and tumorigenesis, respectively. However, the molecular basis underlying the maintenance and differentiation of normal mammary stem cells are largely unknown.

To characterize cells with stem-like characteristics, we determined the gene expression and genetic profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Gene expression profiles were analyzed using SAGE (Serial Analysis of Gene Expression), whereas genetic alterations were investigated using SNP (Single Nucleotide Polymorphism) arrays and FISH (Fluorescence In Situ Hybridization). SNP analysis suggested that CD24+/CD44– and CD24–/CD44+ cells from the same tumor are clonally related, but can be both genetically and epigenetically distinct. For example, CD44+ cells have an activated TGFb signaling pathway, while TGFβ-signaling specifically decreases in CD24+ cells due to increased TGFBR2 promoter methylation. This is consistent with the observation that CD44+ cells respond strongly to an inhibitor of TGFBR, acquire more differentiated epithelial cellular morphology and membrane localization of E-cadherin and β-catenin, while CD24+ cells do not respond to the inhibitor of TGFBR. Furthermore, gene expression profiling identified breast tumors with a higher or lower fraction of CD44+ cells and revealed that lymph node-negative breast cancer patients whose tumors were enriched for CD44+ cells had shorter overall survival as well as shorter distant metastasis-free survival. Interestingly, CD24+ cells appeared to be more prevalent in metastases to distant organs, even when the primary breast tumor was enriched for CD44+ cells. This suggests that the tumor cells may be altered during the metastatic process, or that CD24+ breast cancer cells are intrinsically more competent for metastasis. These studies demonstrate that cancer cell phenotype is subject to dynamic regulation by genetic and epigenetic mechanisms as well as by the tumor microenvironment. Thus, tumor progression is a dynamic and complex process that is influenced strongly by the intrinsic level of genetic instability in a given tumor at a given time and location.