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), genetic alterations were investigated using SNP (Single Nucleotide Polymorphism) arrays and FISH (Fluorescence In Situ Hybridization), and DNA methylation patterns were analyzed using MSDK (Methylation-Specific Digital Karyotyping).

Results: The CD24+ more differentiated and the CD44+ stem cell-like populations from the same tumor were clonally related but not always identical and epigenetically distinct. A gene signature specific for CD44+ cells was enriched for known stem cell markers and was associated with decreased overall and distant metastasis free survival in lymph node negative breast cancer patients. Systemic network analyses determined that the TGF-β pathway is specifically active in CD44+ breast cancer cells and its inhibition induces their epithelial differentiation. CD24+ and CD44+ also demonstrated distinct responses to various therapeutic agents.

Conclusions: Our study demonstrates 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. Understanding the molecular mechanisms responsible for breast tumor heterogeneity and specific targeting of each cell types within tumors will facilitate the development of more effective ways to treat and prevent breast cancer.

Thursday, 17 April 2008 11:00–12:30

KEYNOTE SYMPOSIUM

Tailoring local regional therapy

195 Invited

Arrays – are they helpful?

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Since the late 1990's microarray analysis has been increasingly used to study breast cancer. Most studies so far have either looked at overall gene expression and have tried to subcategorize tumors based on differences in gene expression (unsupervised analysis) [1−3], or have focused on developing of prognostic assay's that predict changes of developing metastasis by linking gene expression data to clinical outcome data (supervised analysis) [4,5]. Clinically, these assays can be used for treatment discussion making, e.g. adjuvant systemic treatment advice. Two assays are currently being tested prospectively in their ability to better predict the need for adjuvant treatment in node-negative breast cancer (respectively the 70-gene prognosis profile in the MINDACT Trial [6] (microarray based) and the 21-gene recurrence score in the TailorX Trial [7] (PCR-based assay)). Another interesting possible clinical application is therapy response. A few studies haven been published on chemotherapy response [8−10].

For breast cancer radiation oncologists the question is: can I expect clinical assays to help guide decision making for post-mastectomy radiotherapy (yes/ or no in clinically intermediate risk patients) and selecting patients that might not be offered breast conservation because of a very high risk of local recurrence or perhaps patients that might benefit from a higher dose (boost). Last but not least, identification of patients with a very low risk of local recurrence that might be offered partial breast irradiation.

Cheng et al. applied a supervised learning approach on a breast cancer patient cohort all treated by mastectomy without post-mastectomy radiation [11]. The identified a 34-gene classifier that could predict local recurrence in the validation set with a sensitivity of 67% and specificity of 83%. Looking at the biology behind this list processes like cell cycle regulation, cell death and proliferation can be found.

In patients treated by breast conserving therapy, age is an independent predictive factor for local recurrence, even after compensating for margin status, radiotherapy dose and estrogen receptor status [12]. By hypothesizing that in optimally treated patients, young age represents specific poor prognosis biology, Kreike et al looked at patients under 51 years of age treated by breast conserving therapy and by definition all patients received radiotherapy. They applied different methods and found one robust list that was presumably driven by ER-status. After correction for ER status, no robust classifier was found.

We applied a different supervised approach on a young patient cohort (<53) treated by breast conserving therapy [13]. A group of 161 patients were divided in a training and validation set. After a standard supervised approach had failed to segregate groups into a low and high risk group for local recurrence, we tested different signatures that previously had been found to predict metastasis free and overall survival in breast cancer patients [4,14,15]. These signatures (the 70-gene prognosis profile, the wound signature and the hypoxia signature) were optimized towards the clinical end point of local recurrence after breast conservation and subsequently validated. One of the three signatures tested, the wound signature, could segregate groups of patients at low or high risk of a local recurrence. In the validation set a large difference in 10-year local recurrence rate (5% vs 29%) was found with a sensitivity of 88% and a specificity of 75%. The classifier was an independent predictor in multivariate analysis.

Assays that are currently being tested in clinical trials have shown the amount of time, money and effort it takes to bring molecular assays into the clinic. For local recurrence prediction the first step now is validation on archived material. Two large trails that are currently being conducted are collecting tumors samples and potentially allow for validation of these assays.

196 Invited

New imaging techniques for tailoring therapy

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In the patient with breast cancer, there are three different scenarios in which imaging studies are used to guide therapy: In the neoadjuvant setting, functional imaging is used to yield surrogate markers to predict and monitor response to therapy of a given tumor. In the pre-operative setting, structural imaging studies are needed to delineate the extent of disease as accurately as possible. For neoadjuvant treatment, many different concepts have been established or are being developed that target at different molecular pathways. Ideally, an imaging tool should be able to provide information on response to specific molecular pathways and it should help predict response and identify response as early as possible. PET has traditionally been perceived as a "functional" imaging tool for response prediction and assessment. New, specific molecular targets are being developed and evaluated in translational research that target at anti-angiogenetic and growth factor receptor signalling pathways. Moreover, functional MR-based technologies like dynamic contrast enhanced MRI, Diffusion weighted MRI, Perfusion MRI, MR based oxygen mapping, and MR spectroscopy have been introduced that appear to exhibit a similar sensitivity for the pathophysiological processes associated with response. For the second scenario, i.e. pre-operative staging of primary or residual breast cancer, MRI has been an established role to guide surgical treatment. The importance of an accurate staging will be even increase with the increasing use of more regional therapies such as intra-operative radiotherapy. This lecture will review recent results and controversies around imaging for tailoring neoadjuvant and surgical therapy.

197 Invited

Tailoring radiotherapy

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Adjuvant radiotherapy (RT) in breast cancer is now widely accepted as the standard treatment after breast-conserving surgery and in many cases after mastectomy.

Nevertheless, several factors may influence both indications and modalities. For example and in case of conservative breast management, the impact of the boost is still under debate in the elderly. In addition, in hormone receptor positive-cancer patients older than 70 years old, one has recently advocated the equivalent benefit of the sole hormonotherapy after surgery. Finally, new molecular surrogate markers are under extensive researches to identify which patients will or not benefit from RT.

In case of mastectomy, only large primitive tumors and node positive patients (>4 N+) received adjuvant RT. The updated meta-analysis has proposed to extend this indication to the 1−3 N+ patients. In addition, specific genes have recently been identified to help tailoring RT treatment.

In all cases, the survival impact of internal mammary chain RT has not been yet proven and its indication is extremely debated.

Concerning prediction of late toxicities, evidence has accumulated in recent years suggestive of a genetic basis for a susceptibility to the development of radiation injury following cancer radiotherapy. We assessed whether patients with severe radiation-induced sequelae (RIS) display both a low capacity of initial normal tissue apoptosis and possess certain single