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15 INVITED

Profiling clinical behaviour of tumours using DNA methylation markers

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Aberrant DNA methylation is the most common molecular lesion of the cancer cell. Neither gene mutations (nucleotide changes, deletions, recombinations) nor cytogenetic abnormalities are so omnipresent in human tumors as DNA methylation alterations. The most studied change of DNA methylation in neoplasms is the silencing of tumor suppressor genes by CpG island promoter hypermethylation, that target genes so relevant as $p16^{INK4a}$, BRCA1 and hMLH1. There is a profile of CpG island hypermethylation according to the tumor type and genes silent by methylation represent all cellular pathways. The introduction of bisulfite-PCR methodologies combined with new genomic approaches is providing us with a comprehensive spectrum of the genes undergoing this epigenetic change across all malignancies. However, we still know very little about how this aberrant DNA methylation "invades" the previously unmethylated CpG island and is maintained trough cell divisions. Furthermore, we should remember that this occur in the context of a global genomic loss of 5-methylcytosine. Initial clues to understand this paradox should be revealed from the current studies of DNA methyltransferases and Methyl CpG binding proteins. From the translational standpoint, we should make an effort to validate the use of some hypermethylated genes as a biomarkers of the disease such as it may occurs with MGMT and GSTP1 in brain and prostate tumors, respectively. Finally, we must expect the development of new and more specific DNA demethylating agents that awake these methyl-dormant tumor suppressor genes and prove their therapeutic values. The expections are high.

16 INVITED

Expression of replication origins firing genes predicts clinical outcome of primary cutaneous melanoma patients

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Gene-expression profiling in human cutaneous melanomas is impaired by the difficulty in getting access to a retrospective collection of frozen tumors. Thus, compared to other solid tumors, gene expression profiling data on human cutaneous melanomas are scarce, and data with prognostic implication are entirely lacking. In order to better understand the progression of this tumor and to identify key genes involved in melanoma prognosis, we correlated gene-expression profiles with clinical outcome in a cohort of 83 patients with primary melanoma of the skin, and applied a multiple random validation strategy to identify genes with a high probability to predict 4-yrs. distant metastasis free-survival. Profiles were also compared in primary melanomas and paired metastases. We identified a signature based on the top 60 genes discriminating between primary melanomas associated with good and poor prognosis. Some of these genes are key-genes in the regulation of replication origins firing, such as *mini-chromosome maintenance (MCM)* genes and *geminin*. Results have been validated at the mRNA and protein levels in independent populations of 17 and 176 primary melanomas with long-term follow-up respectively, showing that the prognostic value of overexpression of replication origins firing genes is independent from thickness, ulceration, age, sex, and anatomic site.

Scientific Symposium
FECS/ASCO – Malignant lymphomas

17 INVITED

Molecular pathogenesis of lymphomas, where do we stand?

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Much progress has been made into our understanding of the pathogenesis of the non-Hodgkin's lymphomas (NHLs), largely due to advances in molecular biology resulting from completion of the first iteration of the human genome project coupled with technical innovation. Genome-wide gene expression profiling (GEP) has provided a landslide of data regarding the molecular pathogenesis of many NHL subtypes. This presentation will focus on 3 specific lymphoma subtypes, including diffuse large B cell (DLBCL), mantle cell (MCL) and follicular lymphoma (FL).
DLBCL is the most common NHL subtype worldwide, accounting for 30–40% of all lymphomas. Clinical and morphological heterogeneity have long been appreciated, but recent GEP studies have helped to elucidate important aspects of pathogenesis. DLBCL is made up of at least 3 major subtypes, including germinal center B cell type (GCB), activated B cell type (ABC) and primary mediastinal (PMBCL). Specific oncogenic mechanisms are associated with these molecular subtypes. For example, the t(14;18) that characterizes about 15% of *de novo* DLBCL is only found in the GCB-type. Both the ABC subtype and PMBCL are characterized by constitutive activation of the NF-κB signaling pathway, providing a potential target for therapy. Moreover, distinguishing these subtypes has clinical relevance, as the molecular distinctions translate into survival differences and a different disease course. PMBCL shares features of GEP in common with classical Hodgkin's lymphoma, a finding that helps to explain some unique clinical features of this lymphoma subtype.
MCL accounts for ~6% of NHLs and has an aggressive clinical course. It is characterized by the presence of the t(11;14) that leads to deregulation of cyclin D1 expression. GEP studies highlight the importance of the proliferation signature, with widely disparate survival characteristics between groups. The relationship between proliferation and a number of oncogenic alterations will be explored. GEP in FL has shown the importance of the microenvironment to the biology and outcome prediction for this tumor. Distinct gene expression signatures that have a profound impact on overall survival in FL appear to be derived from non-neoplastic cells, in particular, reactive T cells and macrophages. Hypotheses can be generated based on this new knowledge that suggests a role for the immune response in FL. These data will be reviewed.
Finally, new techniques that allow high-resolution analysis of the human genome are being applied to NHL clinical samples. When cases with prior GEP are studied, there is an opportunity to begin to explore the relationship between altered gene expression and chromosomal imbalances. These combined approaches provide some insight into the mechanisms of disease progression and clonal evolution in NHLs. More importantly, they help to identify potential new targets for therapy.

18 INVITED

On the way to a new biological molecular prognostic index: The Lunenburg Lymphoma Biomarker Consortium (LLBC)

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Biological prognostic markers in non-Hodgkin's lymphoma (NHL) exist, but have not gained acceptance in clinical practice. Clinical prognostic indices, such as the IPI in the aggressive lymphomas and the FLIPI in the follicular lymphomas, are widely used, in part following their validation in large cohorts of patients from international trials. However, clinical factors represent surrogates for the underlying biology of NHL and as such do not identify potential targets for novel therapies. Important biomarkers have been described in both diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), but require validation and systematic study before they can be accepted for clinical usage.
The LLBC is an international effort with a mission to move risk stratification beyond the IPI. The ultimate goal of this consortium is to validate a list of important biomarkers initially in patients with DLBCL and FL. We aim to standardize the methodology for routine measurement and determine their independent contribution to prognosis using tissue microarray (TMA) techniques and immunohistochemistry. We will take advantage of large numbers of patients from both recent and remote randomised clinical trials from both North America and Europe. Standardization of the reagents and methodology are performed using test TMAs, further establishing the thresholds for determining positivity. Upon achieving this goal, a candidate list of biomarkers will be analysed using recent clinical trials of DLBCL comparing CHOP chemotherapy vs CHOP + Rituximab. Older studies employing CHOP as the standard arm will also be analysed, shedding light on the impact of therapy in determining prognostic marker relevance. Similar studies in FL will also be performed. Our goal is to determine a list of relevant and independent biomarkers that could be used in conjunction with clinical factors in the design of new prognostic models for the NHLs.