Angiogenesis in bronchial dysplasia and angiogenic squamous dysplasia is associated with the development of immature vasculature

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Background: Angiogenic squamous dysplasia (ASD) is a dysplastic lesion of the bronchial airways that is distinguished from other dysplasias by virtue of the presence of characteristic vascular morphology with projection of microvessels into the overlying dysplastic epithelium. These lesions are associated with increased VEGF expression and high microvessel densities (MVD) in comparison to normal bronchial epithelium. In addition, we have recently demonstrated these lesions to be associated with longer periods of persistence in comparison to non-ASD bronchial dysplasia.

Methods: Immunohistochemical stains for CD31 and actin were performed on consecutive sections of bronchoscopically obtained biopsy material. CD31 and actin vessel densities for identical areas within each biopsy were produced by image analysis with collection of area measurements and vessel tags. A microvessel maturation index (MMI) was calculated via division of actin MVDs by CD31 MVDS in comparison to normal bronchial epithelium. In addition, single vessel analyses were performed for microvessel papillary structures in ASD lesions.

Results: The mean MMI for twelve dysplastic lesions was significantly less than that measured for four normal bronchial biopsies (MMI 0.62 vs. 1.02, respectively; p=0.04). When analyzed independently, the vessels that project into the intraepithelial papillae of ASDs showed the lowest MMI of 0.56. This was not statistically different than the MMI for the dysplasia group as a whole but was significantly decreased in comparison to normal MMI (p=0.71 and 0.04, respectively). Incomplete vascular maturation appears to be associated with dysplastic change in the bronchial airways and is most striking in the vascular structures of ASDs.

Conclusions: Angiogenesis in bronchial dysplasia is associated with reduced vascular maturation. Immature vasculature may have an impact on the growth and progression of dysplastic lesions. Increased delivery of pro-tumorigenic factors such as growth promoting factors and mutagenic substances may be facilitated by immature vascular networks. Further analysis of ASD associated vasculature may allow for the identification of angiogenic mediators that control the maturation of microvessels in pre-neoplastic airway disease.

Two wrongs make a right: the use of whole genome amplification for pair-wise genome-wide copy number analysis of limited patient material

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Background: Alterations in genome structure (e.g. amplifications and deletions) have been identified as features of many types of cancer including lung cancer. A major challenge in the molecular study of lung cancer is the limited quantity of tumour tissue available from routine biopsy procedures. To address this challenge, whole genome amplification (WGA) methods utilizing Phi29 polymerase were developed to increase the amount of DNA available for analysis. The high sequence fidelity of these techniques has been investigated using low resolution assays however the use of amplified material for copy number analysis has been questioned due to amplification-induced bias. In this study, we investigate the use of amplified material for genome-wide copy number analysis of limited quantities of patient material.

Methods: Normal lymph nodes from three patients were fresh-frozen in OCT compound for tissue archival. DNA from these sources was extracted and 7ng (~1000 cell equivalents) subject to WGA using the QIagen Repli-G Mini kit to generate over 10,000ng of product (~1.4M cell equivalents). To identify artifacts induced by the amplification technique, pre- and post-amplification samples were hybridized to the Affymetrix GeneChip Mapping 500k SNP and NimbleGen 384k CGH array platforms. Pre- and post-amplification copy number comparisons were conducted to identify amplification-induced copy number differences.

Results: Preliminary copy number analysis of the Affymetrix data from pre- and post-amplification sample pairs has identified more than 700 sites which were commonly over- or under-amplified. 63 of these sites, representing a maximum of 59Mb and often in telomeric regions, were under-amplified. 683 sites, representing a maximum of 685Mb and primarily in GC-rich regions, were commonly over-amplified. Compensation for these reproducible biases can be achieved by comparing amplified samples to amplified samples. Preliminary pair-wise comparisons of amplified samples have shown recapitulation of copy number differences detected in corresponding unamplified comparisons. In the example shown in Figure 1, the unamplified (a) and amplified copy number comparison (c) identified 14 and 89 variants respectively. Both comparisons identified the relative increase in copy number marked by the arrow as the most significant (p<0.0001).

Figure 1: Example of copy number detection using unamplified and amplified pair-wise comparisons

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Conclusions: Preliminary results suggest that Phi29-based whole genome amplification introduces structural biases that may be related to the composition of the underlying DNA sequence. Some of the reproducible biases induced by Phi29-based WGA may be compensated for by amplifying both samples in pair-wise copy number comparisons.

PD2-2-8 Molecular Pathology, Tue, 16:00 - 17:30

The impact of epidermal growth factor receptor gene status on carcinogenesis of small adenocarcinoma of the lung

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Background: Adenocarcinoma is the most frequent histological subtypes of lung cancers and atypical adenomatous hyperplasia (AAH) is considered as a preneoplastic lesion of adenocarcinoma. According to a hypothesis of multistep carcinogenesis, lung adenocarcinoma develops AAH to invasive adenocarcinoma through bronchioloalveolar carcinoma (BAC). Noguchi classified small peripheral lung adenocarcinoma measured 2 cm or less in the greatest diameter into 6 types. Among them, type A, B and C have BAC components. EGFR mutations are frequently detected in never smokers and adenocarcinomas, especially those with BAC features. We investigate EGFR mutations, EGFR gene copy number, and KRAS mutations in AAH and Noguchi’s type A-C and analyzed the association among histological subsets and genetic and clinicopathological factors to clarify the role of genetic alterations on carcinogenesis of adenocarcinoma with BAC component.

Methods: Sixty lesions measured 2cm or less in greatest dimension which were obtained from 48 patients by surgery were studied: 4 AAH, 19 Noguchi’s type A, 15 type B and 22 type C. EGFR mutations were examined using a mutant-enriched PCR assay for exon 19 deletions and L858R exon 21 mutation, and KRAS mutations were examined using a PCR assay for codon 12 point mutations. EGFR copy number was detected by a fluorescence in situ hybridization (FISH) assay.

Results: One lesion of AAHs had EGFR mutations (25%), but there were no KRAS mutations and high EGFR copy number status in AAHs. EGFR alterations had a tendency to increase the positive alteration according to the advance of histological classification, and high EGFR copy number status was significant frequently detected in Noguchi’s type C than AAH-B group including AAH, Noguchi’s type A and B in univariate analysis (Type C versus AAH-B: 31.8% versus 5.3%, P=0.0091). KRAS mutations were detected in 5 lesions (8.3%) among total 60 lesions without statistical correlation with other factors. Multivariate analysis revealed that Noguchi’s type C significantly correlated with larger tumor size (OR=3.61, 95%CI: 1.12-11.6, P=0.031) and high EGFR copy number status (OR=5.94, 95%CI: 1.25-28.3, P=0.025) than AAH-B group.

Conclusions: EGFR mutations occur in the AAH lesion and may influence the carcinogenesis of lung adenocarcinoma. By contrast, increased EGFR copy number may be a late event of tumor development and play a role in the progression of lung adenocarcinoma.

PD2-3-1 Molecular Targets and Prognostic Factors, Tue, 16:00 - 17:30

Prognostic significance and origin of plasma KRAS mutations in patients with non-small cell lung cancer (NSCLC)

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Background: KRAS codon 12 mutations occur in about 30% of non-small cell lung cancer (NSCLC) tissue and are associated with adenocarcinoma histology, poor survival and resistance to erlotinib or gefitinib. In this study, we evaluated the reliability and clinical significance of plasma KRAS mutations in NSCLC patients.

Patients and Methods: 180 Swiss patients with NSCLC were screened for KRAS mutations in plasma and matched peripheral blood mononuclear cells (PBMC) using a combined restriction fragment-length polymorphism and polymerase chain reaction (RFLP-PCR) assay. Survival analysis was performed using the Kaplan-Meier method and the Cox multivariate model. KRAS mutations were validated in a second laboratory by DNA sequencing, using matched plasma, serum, PBMC and tumor tissue.

Results: Baseline characteristics: 69% male, 69% smokers, 86% stage IIIB/IV and 44% adenocarcinoma. Median age at diagnosis was 61 years and median survival was 12 months. Chemotherapy was given to 78% of the patients, 27% had surgical resection and 12% radiation. Mutation screening revealed KRAS mutations in 16/180 (9%) plasma and 0/180 (0%) PBMC samples. Plasma KRAS mutations (P = 0.014), tumor stage (P < 0.001) and surgical resection (P < 0.001) were independent predictors of prognosis in the multivariate model. No significant associations were found between plasma KRAS mutations and baseline characteristics or response to chemotherapy. DNA sequencing confirmed circulating KRAS mutations in 11/15 evaluable cases. KRAS codon 12 sequences matched between blood and tumors in 7/9 evaluable cases.

Conclusions: Plasma KRAS mutations were associated with poor survival and concordant with tumor KRAS mutations. Further studies are warranted to test if plasma KRAS mutations predict resistance to erlotinib or gefitinib in NSCLC patients.

PD2-3-2 Molecular Targets and Prognostic Factors, Tue, 16:00 - 17:30

Amplification Of Epidermal Growth Factor Receptor Gene And Its Prognostic Implication In Surgically Resected Adenocarcinoma Of The Lung

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Background: An increased copy number for the epidermal growth factor receptor (EGFR) gene has been suggested to be a valid marker to predict response of EGFR inhibitors in the advanced stage of lung cancer. However, no clear evidence has been demonstrated as to whether