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 per 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=0.0071 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 pairwise 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