Quantification of biologically relevant vascular phenotypes in human prostate cancer: Automated image analysis using hyper-plexed immunofluorescence

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Abstract

Although anti-angiogenic therapy has emerged as a leading modality in treating human cancer, further improvements in the duration and frequency of clinical responses of various human cancers remain important clinical needs. Maturity of the tumor vasculature has been identified as one of the major determinants of response to anti-angiogenic treatments. We have developed and optimized the application of a quantitative, high-throughput, hyper-plexed, fluorescence imaging technology to refine our understanding of the complexity of vascular phenotypes in various human malignancies. The technology was used to quantify tumor blood vessels and expression of thirteen proteins of known roles in blood vessel biology in single sections from archival primary tumor tissues from 94 prostate cancer patients. CD31 was used to segment vascular objects in each image. CD31 and CD34 endothelial cell staining, SMA pericyte staining, and collagen IV basement membrane staining were used to classify detected vessels using K-means cluster analysis. Segmented vessels were clustered into 2–20 cluster sets, and the reproducibility of vessel classification of each cluster set was determined using the consensus clustering algorithm. A six cluster set that reflected biologically relevant tumor vascular subtypes with high consensus clustering concordance was selected for further analysis. Clusters consistent with different stages of vessel development were obtained, including clusters with CD34 high/SMA low and CD34 low/SMA high profiles, reflecting immature and mature vascular phenotypes. Additional clusters representing phenotypes consistent with transitional vascular developmental stages were also identified. Nine additional proteins involved in angiogenesis were also quantified in each vessel and expression profiles for each cluster were determined. The enrichment of vascular phenotypes in various human malignancies. The technology was used to quantify tumor blood vessels reflecting immature and mature vascular phenotypes. Additional clusters representing phenotypes consistent with transitional vascular developmental stages were also identified. Nine additional proteins involved in angiogenesis were also quantified in each vessel and expression profiles for each cluster were determined. The enrichment of blood vessel clusters was then analyzed for each patient. This revealed differential patterns of vascular maturity phenotypes in the prostate cancer tissue samples analyzed. We have demonstrated that high-throughput, quantitative characterization of vascular maturity phenotypes is feasible in human cancer tissue specimens. Such immunofluorescence-hyper-plex profiling of vascular-related proteins has the potential to illuminate the complex biology of tumor angiogenesis and to enable novel approaches for patient tailoring in clinical trials of anti-angiogenic therapeutics.

Overview: MultiOmyx™ hyper-plexed staining and analytics

Hypothesis: The maturation state of tumor blood vessels is an important determinant of sensitivity to anti-angiogenic agents. Dvorak et al have demonstrated previously that early stage tumor surrogate blood vessels are extremely sensitive to an anti-VEGF agent (VEGF-Trap), while mature vessels are resistant (Sitohy B, Nagy J, Jaminet S-C, et al. Tumor surrogate blood vessel subtypes exhibit differential susceptibility to anti-VEGF therapy. Cancer Res. 2011;71:7021–7028).

Hyper-plexed analysis of tumors using multiple vascular markers can be used to differentiate subtypes of blood vessels. This approach, which we call “angiophenotyping”, may be useful in categorizing which tumors may have the highest proportions of tumor vasculature that is most sensitive to anti-angiogenic therapies.

Cluster Analysis and Vessel Cluster Signatures

Patients Distinguished by Differential Vessel Cluster Enrichment

• We have established that blood vessel phenotyping in human cancer tissues using hyper-plexed immunofluorescence is feasible
• Validation of these findings and extension to additional markers obtained from study of model systems of angiogenesis is currently ongoing
• A robust blood vessel maturity classification may yield insights into anti-angiogenic therapeutic response, and should be examined in this context in order to impact patient outcomes