Schmidt-Kittler, Oleg [23]

Whole-genome analysis of single disseminated tumor cells isolated from bone marrow of breast cancer patients

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Because genomic changes constantly accumulate during tumor progression, the systemic evolution of cancer cells cannot be deduced by analysis of primary tumors and their metastases. To follow the evolution of systemic breast cancer we analyzed single disseminated tumor cells from the bone marrow of patients with different stages of the disease. Disseminated tumor cells can be detected at a frequency of about one tumor cell per one million bone marrow cells by histogenetic markers, and their detection has been shown to be of prognostic relevance. After isolation of the tumor cells we amplified the genome of the single cells using a recently developed polymerase chain reaction technique. Subsequent comparative genomic hybridization revealed gains and losses of specific genomic regions. The comparison of single disseminated tumor cells isolated from patients with late-stage and early-stage disease now enables us to define differentially affected genomic regions. These regions could eventually turn out to be markers for systemically progressive disease and to identify patients that are at risk for metastatic relapse.

Schnitzer, Jan [24]

Vascular oncoproteomics for tIssue-specific targeting and overcoming barriers to drug and gene delivery *in vivo*

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Target discovery through genomics requires extensive bioinformatic processing and high through-put pharmacological screening. Resulting drugs and gene vectors may work in vitro but not necessarily in vivo where cell barriers restrict tissue penetration to desired effector cells. A novel strategy will be described that uses high-resolution proteomic mapping and novel tissue subfractionation techniques to unmask inherent accessible targets that permit tissue-specific pharmacodelivery and overcome barriers to targeted drug or gene delivery in vivo. Molecular maps of vascular endothelium, specifically its caveolae and luminal endothelial cell plasma membranes isolated directly from tissue, reveal considerable vascular diversity between organs and tumors. New tissue-specific accessible targets have been identified on the endothelial cell surface of specific organs as well as tumor angiogenic blood vessels in vivo. Novel monoclonal antibodies have been generated that target the endothelium and caveolae with single tissue retention of up to 89% of the original IV dose in just 30 min. Drug-immunoconjugates permit localized bioefficacy at levels up to 200-fold more than drug alone. Immunotargeting caveolae also facilitates rapid transcytosis in endothelium for delivery to underlying tissue cells in vivo. Many such targets are induced commonly in rat, rabbit, monkey and human tissues including tumors. It appears that our quest for basic knowledge on the function of caveolae and endothelium has translated into new strategies for tissue-specific targeting, including that of the caveolar trafficking pathway, which may have broad applications in site-directed delivery in vivo.

Schubert, Elizabeth [25]

Allelic imbalance in routinely processed breast tumors determined by Affymetrix HuSNP array

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Analysis of allelic loss in archival tumor specimens using array technology is constrained by the quality and quantity of available tissue. A prototype Affymetrix HuSNP array was shown to provide reliable and reproducible assessment of allelic imbalance for 440 single-nucleotide polymorphisms in frozen esophageal tumors (Mei-2000). However, the commercially available Affymetrix HuSNP array (1,494 single-nucleotide polymorphisms) has not been validated for the assessment of allelic imbalance in tumors processed by standard pathology methods. We tested the HuSNP assay in duplicate on breast specimens using both formalin-fixed and frozen, tumor and normal tissue taken from a single patient (16 arrays). We purified tumor cells using bivariate cytokeratin/DNA flow sorting; normal breast served as the constitutive normal. STR typing on three chromosomes validated regions of allelic imbalance. Allele calls from the HuSNP array averaged 95% reproducibility between duplicates and 94% concordance between the fixed and frozen samples. We also tested DNA from the same samples that had been subjected to whole-genome amplification (primer extension preamplification) before array analysis. Although overall signal intensities were lower, the data from this material was reproducible in duplicates and concordant between sample types at rates similar to those for genomic DNA. Results from genomic normal tissue DNA averaged informative (AB) calls at 379 loci over all chromosomes. Although data points were clustered and large segments of chromosomes were not informative by this technique, our data indicated that the Affymetrix HuSNP assay could potentially provide a low-resolution, genome-wide analysis of allelic imbalance in routinely processed pathology specimens.

Schulze, Almut [26]

Analysis of the transcriptional program induced by Raf in epithelial cells

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Activation of the Raf/MAP kinase pathway is a critical event in tumorigenesis induced by *RAS* and other oncogenes, a major role of this signaling system being the regulation of cellular transcription factors. To address the contribution of MAP kinase–mediated transcriptional changes to the transformed phenotype, we used an inducible form of Raf to analyze early changes in the transcription of some 6,000 genes following activation of the kinase in a normal human breast epithelial cell line. Of the more than 200 significant changes in messenger RNA level detected, genes promoting cell proliferation, invasiveness and angiogenesis featured prominently. Some of the most strongly induced genes encoded growth factors of the EGF family: autocrine activation of the EGF receptor was shown to be responsible for the ability of Raf activation to protect these cells from apoptosis induced by detachment of cells from extracellular matrix (anoikis), which is a critical component of the transformed phenotype.