We have used a two-dimensional restriction landmark genomic scanning technique to identify gene amplification events in esophageal adenocarcinomas. We cloned a multicopy DNA fragment from a tumor two-dimensional gel and confirmed genomic amplification encompassing this fragment by Southern blot analysis. The corresponding DNA sequence was searched by BLAST, which allowed the use of an electronic polymerase chain reaction to map the amplicon to 19q12. We then characterized the amplicon using sequence-tagged site amplification mapping, an approach recently developed in our laboratory. DNAs from 65 esophageal and 11 gastric cardia adenocarcinomas and their normal controls were investigated using 11 sequence-tagged site markers neighboring the cloned fragment. The amplicon, spanning 8 centimorgans, was narrowed to a minimal region of 0.8 cM, which includes the cyclin E gene. We assayed 14 expressed sequence tags covering the minimal region for gene overexpression. Both DNA amplification and messenger RNA overexpression were observed in 7 of the 14 expressed sequence tags selected. Among them, cyclin E demonstrated the highest frequency of gene amplification and overexpression in the tumors examined. After analyzing the sequence-tagged site amplification patterns within BAC contig sequences located at 19q12 in the databases, we further defined the core amplified domain to a region 300 kilobases long. We observed amplification of 19q12 in 13.8% of esophageal adenocarcinomas. Our study is the first to map the core amplified domain of the 19q12 amplicon physically to a 300-kb region, and the data indicate that cyclin E is the probable target gene selected by the amplification event at 19q12.

Linder, Stig [39]

Classification of human ovarian tumors using multivariate data analysis of polypeptide expression patterns

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Quantitative molecular variation may be used for the development of methods for tumor classification. We used the statistical concept of principal component analysis to type ovarian tumors. We purified tumor cells from ovarian tumors and subjected them to two-dimensional gel electrophoresis. Using a data set derived from the quantitation of 170 polypeptides, we established a model (learning set) with 22 tumors for classification into three groups (benign, borderline and malignant) and then used 18 tumors to test the model. We correctly classified six of eight carcinomas and three of four borderline tumors. Of six benign lesions, two were correctly classified, three were classified as borderline and one was classified as a carcinoma. It may be possible to classify tumors by their constitutive gene expression profile using multivariate analysis.

Ling, Zhang [40]

Cloning and analyzing 5′ flanking sequences of a tissue-specifically expressed gene derived from mouse nasopharynx

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To construct an animal model of nasopharygeal carcinoma with a nasopharynx-specific regulator, we cloned the gene *YH1,* which is specifically and highly expressed in human nasopharynx and trachea, through scanning high-density gene filtering. A BLAST screen against GenBank showed that this gene is a close homologue to the *PLNUC* gene, which is strictly expressed in mouse embryonic palate, nasal epithelium and lung and in the trachea of the adult mouse. The same result was obtained by using the polymerase chain reaction with reverse transcription. We cloned and sequenced the 5′ flanking sequence of *PLUNC* with a GenomeWalker kit; this new sequence has been submitted to GenBank (No. 225964). Its promoter activity was confirmed by a luciferase report gene test. The core promoter was 200 base pairs upstream from the transcription starting site. We constructed a green fluorescent protein expression vector with the new promoter, and it showed a specific expression profile in a transgenic *Xenopus* model. We are now using this promoter to induce the expression of the *EBV-BNLF1* gene, and we will transfer this vector to mice by microinjection of fertilized pronuclear eggs.

Lingen, Mark [41]

Molecular profiling of anti-angiogenic agents

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Angiogenesis, the growth of new blood vessels from pre-existing ones, is an essential phenotype for tumor formation and progression, and therefore the inhibition of angiogenesis is an attractive means of chemoprevention and chemotherapy. Approximately 20 inhibitors of angiogenesis are under investigation in clinical trials, and other agents with various biological activities have shown great promise. However, the molecular mechanisms by which these agents work are largely unknown. In order to begin to define specific molecular mechanisms of action, we have used complementary DNA microarray technology to determine gene expression profiles of endothelial cells treated with different inhibitors of angiogenesis. We treated human microvascular endothelial cells with either retinoic acid, interferon-α, a synthetic analogue of fungal protein (TNP470), thrombospondin-1, or pigmented epithelium-derived factor for 4 h. We isolated RNA, labeled it and used it to probe nylon microarray filters that contained a total of 9,154 genes (5,295 named and 3,889 expressed sequence tags). Of these, 205 showed threefold or greater induction or repression. Agglomerative clustering of these genes among the five agents tested resulted in 20 clusters, with each agent behaving differently. Principal component analysis of the expression data also revealed a close similarity of expression patterns between thrombospondin-1 and TNP470. Data detailing changes in expression patterns of all the agents will also be presented. This work demonstrates not only the possibility of obtaining potential molecular profiles of anti-angiogenic agents, but also an opportunity for increased efficacy and reduced toxicity during the development of drugs based on these agents.