MOLECULAR CLASSIFIERS FOR GASTRIC CANCER AND NON-MALIGNANT DISEASES OF THE GaSTRIC MUCOSA

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Modern medicine and especially modern pathology were greatly benefited by recent developments that followed the deciphering of the human genome. Today, a variety of high throughput technologies such as cDNA microarray and Tissue microarray opened new venues for the development of molecular-based diagnostic and prognostic tools that can positively impact disease management. Using these technologies, we can investigate the molecular alterations that underline virtually all diseases.

In this presentation, I will discuss the application of the cDNA microarray technology in human cancer. In brief, one can compare samples based on their expression profile and, by doing so; it is possible to identify genes or gene families whose expression is altered in a given group of samples. The candidate genes can then be assayed by conventional cell biology in order to determine whether its altered expression is related to the pathology. In a different strategy, it is possible to search for expression signatures that can be used to build molecular classifiers. Such classifiers should, in an ideal scenario, be able to distinguish between two groups of samples. The advantage of this latter approach is that it allows for the creation of databases and analysis of individual samples with no need for comparative analysis as in the case of expression profile.

Our group is particularly interested in tumors of the stomach and of the esophagus. In the case of gastric cancer, adenocarcinomas represent 95% of the tumors and, in about 80% of the cases, diagnosis occurs at an advanced stage, with positive lymph nodes. As a consequence, five-year survival is observed in only 5-15% of patients. Intestinal metaplasia of the gastric mucosa is considered as an alteration with high risk of malignazation and hence, an important target form screening molecular alterations that could predict for malignant transformation. In the case of esophagus; SCC is the most frequent tumor type but the incidence of adenocarcinomas of the esophagus is increasing dramatically, especially in USA and Europe. As in the case of the stomach, Barrett’s disease represents the transition from normal squamous esophageal mucosa by a columnar epithelium, as a consequence of gastric-esophageal reflux disease and, likewise, is regarded as risk factor for the development of adenocarcinomas.

Using a cDNA array composed of a selected group of genes known to be altered in human cancer, we compared the expression profile of normal gastric mucosa with that of gastritis, intestinal metaplasia and adenocarcinomas. Based on this comparative analysis, we identified some differentially expressed genes during the evolution of gastric cancer according to the model of Pellaio-Correia and also, compared the diffuse and intestinal types of adenocarcinomas. As our main goal, we searched for molecular classifiers that could distinguish samples in a pair-wise comparison. Using Fisher’s linear discriminant analysis, we searched for trios of genes that could precisely separate two classes of samples. Importantly, we could identify samples of intestinal metaplasia with a molecular signature that resembled that of tumor samples.

More recently, we have extended our studies to samples from esophagus and our goal is to determine the correlation between intestinal metaplasia and adenocarcinomas in both organs.

Data from our group related to cDNA microarray technology can be obtained in the following publications:

Proofs and reprints to: Ludwig Institute for Cancer Research - Rua Professor Antonio Prudente, 109 - 4º and. - São Paulo - SP - 01509-010 - BRAZIL - e-mail: lreis@ludwigm.org.br
MOLECULAR CLASSIFIERS FOR GASTRIC CANCER

Slide 1

Can we identify all differentially expressed genes in a cancer tissue?

- Understanding of oncogenesis
- New drug targets
- Molecular markers for Diagnosis and prognosis

Slide 2

Contraction of "Biochips"
Deposition of cDNA fragments onto a solid surface (nylon or glass).

Slide 3

Risk factors for gastric cancer

- Diet: Salt, Nitrates, Smoked Food, Tobacco.
- Genetics: Mutations on CDH1 gene. Polymorphism on IL-1 / ILRA genes

Slide 4

Signal intensity = (?) mRNA level

Slide 5

Carcinogenesis of gastric tumors

Slide 6

Gastric Cancer

- Diagnosis is frequently made at advanced stage:
  10% in situ,
  80% lymph node positive,
  40% peritoneal metastasis,
  33% liver metastasis.
- Overall five-year disease free survival: 5-15%
- Diagnosis: Endoscopy
- Treatment: Surgery (Radical Gastrectomy) 
  (Very) Poor response to radiotherapy or chemotherapy

Slide 7

Our Goal

To develop a molecular-based diagnostic tool for the detection of gastric cancer based on expression signature, using cDNA arrays.

Slide 8
Differentially expressed genes (p_value < 0.0009, Wilcoxon)

Cluster of 99 samples according to their expression profile of 18 genes

Genes with altered expression in NxTI & NxTd

SVD Score for each trio:

Area of "cloud"
Distance between center of the "cloud"
Molecular Classifiers
(Fisher linear discriminant analysis):\)

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*Exhausted search with 85 samples, 372 genes.
9,510,749 possible trials were tested.  

Creation of a database

Molecular Classification

Can we classify them all?

Classifier for NxT
(99 samples)

Classifier for NxT
(99 samples)
Low SVD, No Mistakes
MOLECULAR CLASSIFIERS FOR GASTRIC CANCER

Classifiers for GxT & MxT (99 samples)

Expression profile of gastric and esophageal mucosa samples

Molecular Classification of all 99 samples

Trios:
Nxt=100
GxT=17
Mxt=20
NnxM=52
GxM=04

Perspectives
Can we predict malignant transformation of non-malignant lesions?

Follow-up of patients with Intestinal Metaplasia:
How many patients?
For how long?

Stomach & Esophagus

References