Differential Gene Expression in Colorectal Cancer: Quantitative Analyses of Angiogenesis Genes and Identification of Potential Candidates as Molecular Markers


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Summary

Introduction
Colorectal cancer is currently one of the most common cancers in Malaysia. The progression of healthy colon tissues to cancer is due mainly to mutations in several genes and occurs in a step-wise manner. Microarray data analyses have revealed the diversity of gene expression within pathologically identical cancers as well as different types of cancers and provide investigators with potentially novel therapeutic targets and molecular markers for cancers.

Aim
- To obtain a differential gene expression profile of colorectal cancer cells in a Malaysia population from microarray data.
- To validate results from microarray data analyses using real-time PCR on tissue specimens from a local hospital.
- To identify potential candidate genes as molecular markers for colorectal carcinoma.

Materials and Methods
Microarray Data Analysis: Microarray data has led to the identification of several genes that displayed unique expression profiles in colorectal cancer. These results have been corroborated with real-time RT-PCR experiments.

RNA Extraction: Healthy and colorectal adenocarcinoma tissues were provided by the University Malaya Medical Centre (UMMC), Kuala Lumpur. Total RNA was extracted from 10 healthy and 10 colon adenocarcinoma tissues using the RNeasy RNA extraction kit from Qiagen.
Real-time PCR: The LightCycler 2 system from Roche Diagnostics was used to perform real-time RT-PCR experiments with SYBR Green I as fluorescent dye. Primer sequences for genes of interest were obtained from PrimerBank database available online at Massachusetts General Hospital server. b-actin was selected as internal control.

Results and Discussions

The expression of Cyr61, a potential angiogenesis factor, was found to decrease up to approximately 4.5-fold while another angiogenesis factor, VEGF, was inconclusive although several samples showed slight up-regulation. These results had hinted at the possibility of antagonistic VEGF-Cyr61 interaction. Microarray data analyses indicated an increased level of expression of the human lysozyme encoding gene, LYZ, and previous research using immunohistochemistry techniques have shown high levels of lysozyme exclusively in colorectal cancer tissues. This was substantiated using real-time RT-PCR where we found up to 440-fold increase in expression levels of LYZ in colorectal adenocarcinoma tissues making it a potential molecular marker for colorectal carcinoma. Microarray data analysis and real-time RT-PCR showed significant decrease in APOE gene expression suggesting its putative role in tumorigenesis of colorectal cancer associated with high fat-intake diet. The expression of ING4, a candidate tumor suppressor gene in brain cancers, was discovered to be suppressed in healthy colon tissues but slightly up-regulated in colon cancer tissues, inferring that ING4 may not play a tumor-suppressing role in the tumorigenesis of colorectal cancers.

Conclusion

- The substantial relative increase in LYZ expression in all the colon carcinoma specimens studied suggests the possibility of utilizing LYZ as a potential molecular marker for colon cancer.
- There is possibly an antagonistic interaction in the expression of the angiogenesis factors Cyr61 and VEGF in colon carcinomas.
- APOE gene may play a role in the mechanism of tumorigenesis associated with high calorie-intake diet.
- ING4 may not play a tumor suppressor role in colon carcinomas.