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Osteopontin identified as colon cancer tumor progression marker

Deepak Agrawal [a], Tingan Chen [b], Rosalyn Irby [b], John Quackenbush [e], Ann F. Chambers [d], Marianna Szabo [c], Alan Cantor [f], Domenico Coppola [c], Timothy J. Yeatman [b,*]

[a] *Department of Cell Biology, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL, USA*
[b] *Department of Surgery, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL, USA*
[c] *Department of Pathology, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL, USA*
[d] *The Institute for Genomic Research, Rockville, MD, USA*
[e] *London Regional Cancer Centre, University of Western Ontario, Ontario, Canada*
[f] *Department of Biostatisitics and Interdisciplinary Oncology, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL, USA*

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Abstract

Identifying molecular markers for colon cancer is a top priority. Using a pooled sample approach with Affymetrix GeneChip technology, we assayed colon cancers derived from a series of clinical stages to identify molecular markers of potential prognostic value. Of 12 000 genes assessed, osteopontin emerged as the leading candidate tumor progression marker. Osteopontin is a secreted glycoprotein known to bind integrins and CD44. Its actual molecular function remains elusive but its increased expression correlates strongly with tumor progression. ***To cite this article: D. Agrawal et al., C. R. Biologies 326 (2003).***

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Résumé

L'ostéopontine identifiée comme marqueur de la progression des cancers du côlon. L'identification de marqueurs du cancer du côlon est hautement prioritaire. En utilisant des pools d'échantillons et la technologie des puces Affymetrix GeneChip, nous avons étudié des cancers du côlon à différents stades cliniques pour identifier des marqueurs moléculaires de valeur pronostique potentielle. Parmi les 12 000 gènes analysés, celui codant l'ostéopontine a émergé comme marqueur de choix pour la progression tumorale. L'ostéopontine est une glycoprotéine sécrétée connue pour lier les intégrines et CD44. Sa fonction moléculaire réelle reste inconnue, mais l'augmentation de son expression se corrèle fortement avec la progression tumorale. ***Pour citer cet article : D. Agrawal et al., C. R. Biologies 326 (2003).***

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* Corresponding author.
E-mail address: yeatman@moffitt.usf.edu (T.J. Yeatman).

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1. Introduction

Few clinically useful tumor markers have been identified for the management of human colon cancer [1]. New tumor markers and markers of tumor progression are needed for improved staging and treatment of many cancers. At present, despite a large number of potential markers of cancer having been identified, carcinoembryonic antigen is the only tumor marker that has gained widespread clinical use in the management of human colon cancer. Its use, however, is marred by its lack of expression in a significant number of cancers and its lack of correlation with tumor response to therapy. Gene expression profiling techniques offer the opportunity to discover new molecular markers [2,3]. We hypothesized that tumor markers and markers of tumor progression could be rapidly identified using a pooled sample approach. By pooling RNA derived from tumor samples of the same clinical stage, we proposed that markers common to the majority of tumors used to derive the pool could be identified.

2. Experimental methods

To validate the capacity of sample pooling to permit the identification of tumor markers common to the majority of patients, total RNA was extracted from five microdissected Astler–Collier stage C tumors and assessed individually. Next, their physical pool was interrogated using Affymetrix HuFl 6800 GeneChips. The results of these microarray assays between the individual samples and their physical pool were then directly compared such that correlation coefficients could be derived, based on gene-by-gene comparisons across tumors. Individual tumors were compared with (1) their physical pool, (2) their mathematical average (calculated pool), and (3) a second pool of five Astler–Collier stage C tumors (completely different from the first set of tumors). A novel algorithm was then derived to improve the results of the standard Affymetrix analysis algorithm. The new algorithms eliminated negative mismatch pairs when the perfect match oligonucleotide intensity was less than the mismatch oligonucleotide intensity. To validate the pooling concept, 11 genes were selected at random for a list of over- or under-expressed genes predicted by the first pool of tumor samples. Northern analyses were performed on these 11 genes using four paired normal and tumor samples derived from an independent set of Astler–Collier stage C tumors. After the pooling concept was validated, we sought to derive *tumor progression* markers from pooled sets of tumors grouped by clinical stage. Total RNA from human colon tumors ($n = 60$) of multiple stages (adenomas, cancers with modified Astler–Collier [AC] stages B, C, and D, and liver metastases) were pooled within stages, and compared with pooled normal mucosal specimens ($n = 10$) by use of Affymetrix 6800 and 12 000 oligonucleotide expression arrays. Hybridization data were analyzed using an algorithm we developed to eliminate negative data and used to infer changes in gene expression, focusing on genes that showed consistent increase or decrease in their expression through tumor progression. All statistical tests were two-sided.

3. Results and discussion

More than 300 candidate tumor markers and 100 markers of tumor progression were identified and eleven were validated by northern analysis [4]. Tumor markers were derived from the pooled Astler–Collier stage C tumors. Tumor progression markers were derived from the comparison of pooled tumors of multiple Asler–Collier clinical stages. The sample pooling approach permitted these observations with the use of relatively few GeneChips and little data analysis. By comparison, others have reported results derived from the use of many more GeneChips on large numbers of individual patient samples. A pooled approach permits the rapid identification of markers common to the samples used to construct the pool but prohibits the detection of gene expression variability between individuals. The gene most consistently differentially expressed in conjunction with tumor progression was that of the secreted, integrin-binding protein, osteopontin. Its potential as a progression marker was validated (Spearman's rho = 0.903; $P < 0.0001$) with northern analysis using RNA from an independent set of normal ($n = 10$) and tumor samples representing all stages ($n = 43$). Moreover, a statistically significant correlation between osteopontin *protein* expression and advancing tumor stage was identified using

303 specimens (human cancer = 185, adenomas = 67, and normal mucosal specimens = 51) (Spearman's rho = 0.667; $P < 0.0001$). In addition, we determined that osteopontin expression was common to a large set of human cancer types, with the exception of brain cancers and hepatomas.

4. Conclusions

We demonstrated that sample pooling could be a powerful, cost-effective, and rapid means of identifying the most common changes in a gene expression profile. We identified a large number of candidate tumor markers and tumor progression markers. Osteopontin emerged as the leading candidate, amongst approximately 12 000 named genes, for a clinically useful marker of tumor progression by use of gene expression profiling on pooled samples. Fortuitously, osteopontin is a secreted marker that is detectable in human sera. For this reason, there is promise for osteopontin as a potentially useful clinical marker of tumor progression or metastasis.

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