The identification of metastasis-related gene products in a rodent mammary tumour model.

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Breast cancer is currently the most common form of serious malignancy in women, affecting approximately 1 in 9 of the female population in the Western World [1]. The etiological background of breast cancer is currently largely unclear; however, diet, reproductive lifestyle and heredity are thought to be important risk factors [2]. The most life-threatening aspect of many forms of malignancies, including breast cancer, is the ability of cells from the primary tumour to disseminate to distant sites of the body. This dissemination, or metastasis as it is known, is a complex multistep process requiring a number of genetic interactions and a variety of gene products [3].

In order to study the process of metastasis at the molecular level, a rodent mammary tumour model has been developed in our laboratory. This completely syngeneic system centres around the use of the highly-inbred Furth-Wistar rat [4]. A cell line, designated Rama 37 was isolated from a benign mammary tumour that developed following treatment of the Furth-Wistar rat with dimethylbenz[a]anthracene (DMBA). This cell line, when injected into the mammary fat pads of the syngeneic host with dimethylbenz[a]anthracene (DMBA). This cell line, when injected into the mammary fat pads of the syngeneic host produces benign, nonmetastatic, encapsulated tumours. A metastatic derivative of the Rama 37 cell line has previously been produced by transfecting this cell line with genomic DNA fragments from a metastasizing human breast carcinoma -derived cell line [5]. The resultant transfected cells, when injected into the mammary fat pads of the Furth-Wistar rat, developed metastases at a high incidence. From one such lung metastases, a cell line was isolated designated Ca2-5-LT1 which when introduced into the host also metastasized [5].

In an attempt to identify those gene products that are associated with the progression from the benign tumour producing cell line, Rama 37 to the metastatic derivative, Ca2-5-LT1 a variation of the subtractive hybridisation technique has been used [6-7]. This technique was performed in a manner that permitted the identification of those mRNAs expressed more highly in the Ca2-5-LT1 cell line when compared to the Rama 37 cell line. Following the implementation of the subtractive hybridisation procedure and the production of a subtracted cDNA library, Northern blot analysis was performed using individual subtracted cDNAs as hybridisation probes. From these studies a number of subtracted cDNAs were identified which corresponded to mRNAs that were expressed more highly in the metastatic cell line when compared to the non-metastatic counterpart. The nucleic acid sequence of these cDNA molecules was then determined and compared to the cDNA sequences stored on the Genbank data base (Table 1).

Table 1: Identification of subtracted cDNAs corresponding to differentially expressed mRNAs.

<table>
<thead>
<tr>
<th>cDNA clone</th>
<th>Identity</th>
<th>% homology</th>
<th>cDNA size (kbp)</th>
<th>Relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopontin</td>
<td>99.4</td>
<td>1.5</td>
<td>9.4</td>
<td></td>
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<tr>
<td>Transaldolase</td>
<td>84 + 85</td>
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<td>6.1</td>
<td></td>
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<tr>
<td>Osteopontin</td>
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<td>0.6</td>
<td>9.3</td>
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<tr>
<td>Transaldolase</td>
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<td>6.1</td>
<td></td>
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<tr>
<td>L17 ribosomal subunit</td>
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<td>0.7</td>
<td>7.2</td>
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<tr>
<td>Osteopontin</td>
<td>100</td>
<td>0.9</td>
<td>9.2</td>
<td></td>
</tr>
</tbody>
</table>

a Identity based on greatest nucleic acid sequence homology to known rat cDNAs.

b Degree of nucleic acid sequence homology between the subtracted cDNA clone and the sequence of the known cDNA.

c Level of mRNA in the Ca2-5-LT1 cell line relative to its level in the Rama 37 cell line. Levels were standardised with respect to a constitutive hybridisation probe [8].

d There are 2 distinct regions of homology between cDNA clones 2 and 4 with the cDNA for human transaldolase. The 2 regions correspond to a 84% and 85% homology, respectively. There is currently no rat transaldolase cDNA sequence available in the Genbank database.

The potentially most interesting subtracted cDNA which corresponds to a differentially expressed mRNA is the cDNA representing osteopontin (OPN). OPN has previously been implicated in metastasis in other studies [9]. Furthermore, in our study cDNAs corresponding to OPN mRNAs represented approximately 20% of all the cDNAs in the subtracted library. This would suggest that the increased expression of the mRNA for OPN is one of the major changes in the levels of specific mRNA species between the metastatic Ca2-5-LT1 and benign Rama 37 cell lines. It is of course important to remember that differential expression of an mRNA between these 2 cell lines is not by itself sufficient evidence to suggest that these mRNAs are directly associated with metastatic phenotype of the Ca2-5-LT1 cell line. It is possible that these mRNAs may represent merely passenger changes that are essentially independent of the metastatic process. Therefore, future studies will require further analysis of these gene products to assess any potential role in metastasis.

References: