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doi:10.1136/jmg.2008.063123

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Germline mutation of microRNA-125a is associated with breast cancer

W Li,1 R Duan,1 F Kooy,2 S L Sherman,1 W Zhou,3 P Jin1

ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that inhibit expression of specific target genes at the posttranscriptional level. MiRNAs are often found to be misregulated in human cancer, and they can act as either potent oncogenes or tumour suppressor genes. Here we show that a germline mutation in mature miR-125a is highly associated with breast cancer tumorigenesis, suggesting that miR-125a is likely to function as a tumour suppressor gene in human cancer.

Breast cancer is one of the most frequent malignancies affecting women,1,2 who run about a 10% cumulative lifetime risk of developing this disease.1 Genetic susceptibility to breast cancer in women is conferred by a large number of genes, of which six have been identified so far. In the context of multiple case families, BRCA1 and BRCA2 are of most importance.3 Mutations in these genes are linked to high lifetime risks of breast cancer and ovarian cancer, as well as moderate risks of prostate cancer and some other cancer types. Mutations in the CHEK2 and ATM genes, by contrast, cause a much more modest rise (2–4 fold) in the risk of developing breast cancer. To date, the genes identified explain approximately 20% of the familial aggregation of breast cancer; the remaining susceptibility genes have proven elusive. Breast cancer tumorigenesis can be described as a multistep process in which each step is thought to correlate with one or more distinct mutations in major regulatory genes. Oncogenes that have been reported to play an early role in breast cancer are MYC, CCND1 (Cyclin D1), ERBB2 (HER2/neu), and ERBB3.4

MicroRNAs (miRNAs) are 18 to 25 nucleotide (nt), single stranded non-coding RNAs that are generated from endogenous hairpin shaped transcripts.5 MiRNAs can suppress posttranscriptional gene expression by base pairing with their target messenger RNAs (mRNAs) and inducing either translational repression or mRNA degradation.5 MiRNAs are initially transcribed as long primary transcripts (pri-miRNAs), which are then processed into ~65 nucleotide hairpin shaped precursor miRNAs (pre-miRNA). Pre-miRNAs are further cleaved to produce mature miRNAs.6 MiRNAs regulate a wide range of biological processes in development and human disease.6 A strong link between miRNAs and cancer has been demonstrated recently, opening up a new avenue of investigation for cancer biology.7,8 MiRNA expression profiling could both faithfully reflect developmental lineages and classify disease states. It has been shown that miRNAs are aberrantly expressed in human breast cancer, supporting a role for miRNAs in breast cancer tumorigenesis.8–10 However, to date no mutations within the miRNA genes have been connected with any human diseases.

Here we show that a germline mutation in mature miR-125a is highly associated with breast cancer tumorigenesis, suggesting that miR-125a is likely to function as a tumour suppressor gene in human cancer.

PATIENTS AND METHODS

Study population

The cohort of breast cancer patients consisted of lymph node negative breast cancer patients who had received loco-regional treatment consisting of mastectomy or tumorectomy followed by radiation therapy for T1-2N0M0 breast cancer at the University Hospital Antwerp and the General Hospital Saint-Augustinus, Antwerp, Belgium between 1986 and 1992. These patients had undergone complete axillary lymph node dissection for nodal status assessment. The original patients were described previously.11 12 Two control groups were obtained from the USA and Europe. There were 587 Caucasian control individuals collected in the USA by SLS; 282 controls were collected from the general population in the Antwerp area by FK.

DNA collection and genotyping

DNA from both adjacent normal breast and tumour tissues of the breast cancer cohort were isolated and subjected to whole genome amplification. TaqMan single nucleotide polymorphism (SNP) genotyping was performed as described previously. For each sample, two independent analyses were performed, and the ones that consistently indicated the presence of a T allele were further confirmed by direct DNA sequencing.

RESULTS AND DISCUSSION

Sequence variations, including SNPs, could potentially influence the processing and/or target selection of miRNAs. In our previous study we identified one miRNA SNP associated with a mature form of miR-125a that significantly blocks miRNA biogenesis.13 Through a series of in vivo analyses, we showed that this miR-125a SNP significantly blocks the processing of pri-miRNA to pre-miRNA, in addition to reducing miRNA mediated translational suppression.10 MiR-125a was also found to be downregulated in several breast cancer miRNA profiling studies.8 10 Furthermore, it has also been demonstrated that overexpression of miR-125 could suppress the
dependent growth and exhibited reduced migration and cell anchorage suppression of both ERBB2 and ERBB3 signalling, breast cancer DNAs from both adjacent normal breast and tumour tissues heterozygous genotypes were confirmed by direct DNA assays, we found no individual carrying the T allele in either control collection, suggesting that the T allele is a mutation. Furthermore, since no individual from the Antwerp control group carries the T allele, we conclude that the presence of the T allele among breast cancer patients from the same area is unlikely due to a founder effect in this population. These results together suggest that germline mutation in mature miR-125a is highly associated with breast cancer tumorigenesis.

Our data indicate that miR-125a is likely to function as a tumour suppressor gene in human cancer. The loss or reduction of miR-125a could lead to the upregulation of miR-125a target mRNAs, such as ERBB2 and ERBB3, and predispose individuals carrying this allele to breast cancer. It will be important to examine further the expression of miR-125a in the tissue samples derived from the patients carrying this mutation, and determine the protein levels of ERBB2 and ERBB3 in these individuals. To our knowledge, this is the first mutation within the miRNA genes found to be associated with human diseases. We believe further resequencing on all known miRNAs in different disease states is now warranted.

Acknowledgements: We would like to thank S Warren, S Chang, and C Strauss for their helpful discussions and critical reading of the manuscript.

Funding: PJ is supported by NIH grants (NS051630 and MH076090). PJ is the recipient of a Beckman Young Investigator Award and a Basil O’Connor Scholar Research Award and is an Alfred P Sloan Research Fellow in Neuroscience.

Competing interests: None.

Patient consent: Obtained.

REFERENCES


![Figure 1](A) The hairpin structure of miR-125a precursor RNA is shown. Mature miRNA is highlighted in blue, and the polymorphic nucleotide is in red. (B) DNA sequencing analyses of controls and individuals carrying the T allele in a collection of 72 breast cancer patients are shown.

Table 1 Minor T allele of mature miR-125a is associated with breast cancer

<table>
<thead>
<tr>
<th></th>
<th>G/G</th>
<th>G/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer patients from Antwerp, Belgium (72)</td>
<td>66 (91.7%)</td>
<td>6 (8.3%)</td>
<td>0 (0%)</td>
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<tr>
<td>Controls from Antwerp, Belgium (192)</td>
<td>192 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Caucasian controls (587)</td>
<td>587 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CEPH diversity panel (1200)</td>
<td>1063 (99.91%)</td>
<td>1 (0.09%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

CEPH, Centre d’Etude du Polymorphisme Humain.


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