

Identification of differentially expressed sense and antisense transcript pairs in breast epithelial tissues

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Introduction

- 20% of human transcripts have naturally occurring antisense products.
- Naturally occurring antisense transcripts were identified using large collections of mRNA sequences, genomic and EST sequences, as well as expression data such as serial analysis of gene expression and massively parallel signature sequencing (MPSS).
- In both human and mouse genomes more than 10,000 potential sense/ antisense transcript pairs (SAS pairs) have been identified.
- Several antisense containing databases such as antiCODE have already been published.
- A discrete function of SAS pairs in human tissues has not been identified, some of which may play a key role in a range of human diseases.

Aims

While the majority of studies have focused on the mapping and evolutionary aspect of NATs and SAS pairs, only a few studies have interrogated and validated their abundance in different human tissue. Our aim was to investigate comprehensively the differential expression levels of SAS pairs between a normal and a malignant in the breast epithelium.

Comparison of detectable sequences between Affymetrix HG U133 Plus 2.0, Almac Cancer DSA and MPSS

	DIRECT ORIENTATION			INDIRECT ORIENTATION		
	Affymetrix	Almac	MPSS	Affymetrix	Almac	MPSS
Affymetrix	21,078 HTR (38,047 -69%)			2,995 HTR (3,476 -6%)		
Almac	16,459 HTR	17,737 HTR (33,355 -54%)		1,753 HTR (8,426 -14%)	6,358 HTR	
MPSS	7,856 HTR	7,878 HTR	8,452 HTR (13811 -69%)	51 HTR	101 HTR	203 HTR (215 -1%)

Figure 1. Probesets from both microarray platforms, as well as MPSS tags, were mapped by sequence alignment to a human transcriptome (HTR) database. Stringent filtering criteria were applied to the probe sets and MPSS tags; first, probe sets and MPSS that could not be mapped to a HTR cluster at all or which showed cross-hybridisation to several clusters were eliminated; second, probe sets had to exclusively detect either the sense or antisense transcript. Direct orientation indicate sense transcripts, whereas indirect orientation represent antisense transcripts coming from the Affymetrix HG U133 Plus 2.0 GeneChip®, Almac Diagnostics Breast Cancer DSA and from massively parallel signature sequencing (MPSS).

Higher detection rate of sense and antisense transcripts with a tissue specific microarray

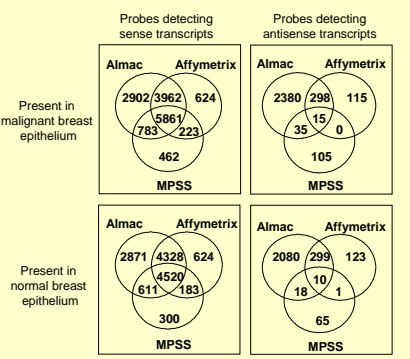


Figure 2. To determine which of the sense and antisense strand-matching probes showed expression in human breast epithelium, the absent or present calls for all probe sets on both microarray platforms in either the normal luminal epithelial or the malignant breast epithelial sample were established using the MAS5 algorithm. Microarray features were included for further studies if at least two technical replicates per platform agreed in their present calls, while MPSS tags were kept when their tag count was at least 3 tags per million. Both were represented by their corresponding HTR cluster identifier.

163 SAS pairs expressed in the breast epithelium

HTS Cluster ID	Cluster Size	Sense	Antisense	Alignment Position	Alignment M value	Sense probe	Alignment Position	Sense M value
HTR02209	unknown	BRAD_4303_a	28939-29398	4,86133062	BRAD_20114_a	28541-29341	2,81474143	
HTR02077	STC1	BRAD_1786_a	12235-12915	2,01831928	BRMS_1886_a	12235-12985	1,22205695	
HTR03709	JUN	BRAD_17225_a	9485-10066	2,80988828	BRM1_4732C12_a	9214-9212	2,94644179	
HTR03742	MALAT1	BRAD_30023_a	6584-1058	2,63871082	BRMNC_2833C12_a	7050-1051	1,82432188	
HTR02073	TRIM59	BRAD_1888_a	138237-13107	2,40624472	HM4659-3233C142_a	13787-13138	2,38820003	
HTR04293	SIRT1	BRMNC_1787C12_a	58241-60566	2,32705669	BRM1_7262C12_a	58200-61742	2,32688417	
HTR02590	SAT1	BRAD_31461_a	1519-1871	2,12615555	BRM1_1320C12_a	1280-1729	1,70388685	
HTR02028	PCNA2	BRAD_32271_a	12771-12521	1,97924857	BRM1_1204H191_a	12771-12515	2,41382209	
HTR02081	LIF1	BRAD_32221_a	22589-60556A	1,87894469	BRM1_3207L_a	20502-20520	2,41323268	
HTR02113	MMP24	BRM1_2823C12_a	57338-67804	1,80095844	BRM1_2823C12_a	56534-57720	1,80407387	
HTR03742	MALAT1	BRAD_20231_a	4236-4881	1,79902321	BRAD_20118_a	3990-4390	1,69152472	
HTR03685	NOT3	BRMS_2387_a	24239-240710	1,14460285	BRAD_20146_a	24234-24209A	1,08838668	
HTR03073	AAK12	BRAD_32008_a	17773-11820A	1,19649555	HM4611-12114_a	11724-11842A	1,120110319	
HTR02728	SLC39A8	BRAD_181742_a	18709-11872A	1,30277436	BRPD_1532C12_a	18240-18720	1,4826382	
HTR02072	SPPL2L	BRAD_20377_a	546-1158	1,38207688	BRMS_1914_a	4430-4280	1,07464645	
HTR03073	AAK12	BRAD_4534962_A_a	117564-11945A	1,50519528	BRM1_3445402B_A_a	11724-11823	2,24879718	
HTR05624	NPAL3	BRAD_30384_a	58627-6521A	1,57541185	BRMS_16276_a	58564-55548	2,28526220	
HTR04895	IRF82	BRM1_841_A_a	28937-29498	1,68193024	BRAD_148L_a	28788-29370	1,67432444	
HTR03685	DCC3L2	BRAD_1201986_A_a	22816-22850	2,00418819	HM4610-3234C12_a	22835-22856	2,32716219	
HTR01040	unknown	HM231823C12_a	53316-54415	2,37021674	BRAD1_2004552_a	53376-54124	1,522770219	
HTR02022	DCBLD2	BRAD_31134_a	104786-102326	2,511911421	BRMS_12648_a	102233-10252A	2,38411207	

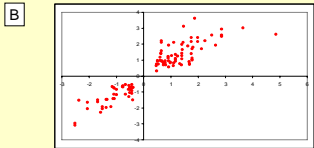


Figure 3. 431 antisense transcripts were identified in the normal and malignant breast epithelium. Validation of the existence of an antisense transcript was defined if the antisense transcript was either expressed in both normal and malignant samples or in at least one sample and seen by at least two platforms. 257 antisense transcripts had a corresponding sense transcript, of which 94 SAS pairs were found in a database established by Galante *et al.*, 2007. 163 SAS transcripts of such pairs have not been previously reported and the top 10 differentially expressed SAS pairs are listed in A. Correlation analysis of SAS pairs expression using Pearson correlation (B).

Location of sense and antisense probe sets

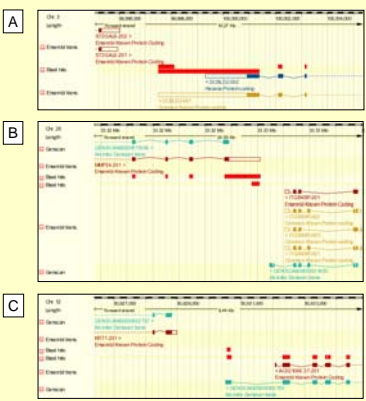


Figure 4. Genome browser for DCBLD2 (A); MMP24 (B) and KRT81 (C), indicating probe sets in sense and antisense orientation, respectively.

Confirmation of sense and antisense expression in breast cancer cell lines by strand-specific qPCR

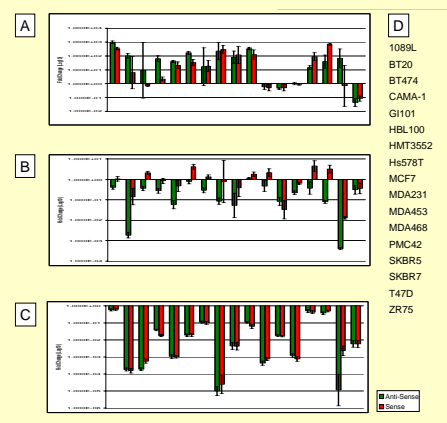


Figure 5. Fold changes in expression of a selected group of SAS pairs were performed by qPCR, using the $\Delta\Delta CT$ relative quantification method. 100ng of RNA was used to generate two independent cDNA syntheses for all samples using Omniscript Reverse Transcription Kit (Qiagen, UK) as per manufacturer's guidelines. Primers for reverse transcription were designed to be gene and strand specific for both the sense and antisense strand (PrimerDesign Ltd, UK) for DCBLD2 (A), MMP24 (B) and KRT81 (C). Analysis of qPCR data was performed using the immortalised luminal cell line (Z26L) as comparator for all the breast cell line (listed in D). ACTB was used as endogenous control throughout all analysis. $\Delta\Delta CT$ relative quantification data are expressed as mean fold changes across samples together with 95% confidence intervals.

SAS expression in primary breast cancers

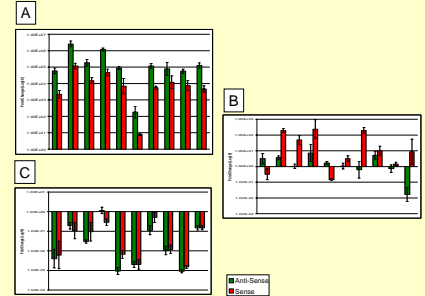


Figure 6. The expression of DCBLD2 (A), MMP24 (B) and KRT81 (C) SAS pairs in 10 primary breast cancers substantially enriched for the neoplastic epithelial component was evaluated, using quantitative strand-specific qPCR. qPCR was performed as described above.

Summary

- More than 2500 antisense transcripts were detected in normal luminal cells and in primary breast tumors substantially enriched for epithelial cells.
- 431 natural occurring antisense transcripts were confirmed by either of the other two technologies.
- Out of those 163 clusters that contain SAS pairs could not be found within a publicly available database (Galante *et al.*, 2007).
- Orientation specific qPCR of selected SAS pairs validated their expression in several breast cancer cell lines and solid breast tumours.