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Short communication

Differentially expressed genes of reprogrammed human pluripotent stem cells in breast cancer

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ABSTRACT

Reprogramming of human somatic cells into pluripotent cell types gives insight in the pathophysiology of diseases. We analysed genes recently shown to be differentially expressed in induced pluripotent stem cells (iPS) in 95 breast cancer samples. This analysis reveals two breast cancer subgroups with stem cell-like features, differing in ER-status and proliferation as well as in their clinical course of disease.

1. Introduction

To date, several hypotheses for the development and growth of breast cancer exist. One of the most interesting is the stem cell model, since it conjoins biology, anatomy and specific disturbances in cell compartments of the breast. However, it is still unclear which role mammary epithelial stem cells play in terms of tumour development and growth. There is emerging evidence that in a malignant bulk tumour a small proportion of 'tumour stem cells' exist, leading to tumour growth by their proliferative activity. In contrast the 'maturation arrest theory' proposes that genetic alterations leading to malignant transformation can occur in specific cellular compartments as, e.g. mammary epithelial stem cells, progenitor cells or differentiated cells (myoepithelial or ductulo-lobular cells) resulting in a block of further differentiation and development of bulk tumours sharing phenotypic properties of the initial cell type of origin.

In the 30th November 2007 issue of Cell, Takahashi and colleagues[1] could demonstrate that the transfection of four genes (*Oct3/4*, *Sox2*, *c-Myc* and *Klf4*) in differentiated human fibroblasts (HDF) results in a conversion to an undifferentiated cell type, which shares properties of pluripotent stem cells (induced pluripotent stem cells or 'iPS'). Based on these cells, the authors were able to induce a directed differentiation into neural and cardiac cells. The global characterisation of such iPS especially by gene expression analysis could provide new insights in the biological properties of proposed mammary epithelial stem cells and the development of breast cancer, because likewise it would be conceivable to induce mammary epithelial cells from iPS.

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2. Cluster analysis of breast cancer samples by using genes differentially expressed in iPS results in biologically and clinically meaningful subgroups

In their report, Takahashi and colleagues performed microarray analysis to investigate the differential expression of genes in iPS compared to HDF. The authors identified 3583 genes, which were up- or downregulated more than fivefold. To investigate the transcriptional diversity and to analyse the function of stem cell differentiation markers in breast cancer, we performed a hierarchical cluster analysis of $n = 95$ breast cancer samples, using those 3583 differentially regulated genes (Fig. 1A). Three major components were observed representing known stem cell markers (as, e.g. *CD133*,[2] *KIT*,[3–5] *NDRG2*,[6] *FZD7*,[7] *TM4SF1*[8] and *PODXL*;[9] Fig. 1B) as well ER-associated genes, and markers correlated with proliferation (Fig. 1C). Two gene clusters were associated with proliferation (Fig. 1C). First, a series of well-known markers were involved in the cell cycle machinery (upper part of Fig. 1C; e.g. *BUB1*, *MCM*-2, -3, -6, -10, *CDC25A*, *CDC6*, *AURKB* and *TTK*), which are strongly correlated with the proliferation marker Ki67. A second cluster of genes was characterised by an inverse pattern of expression in most samples (lower part of Fig. 1C). Amongst those genes which show mostly low expression in tumours with high proliferation, we observed several known markers for myoepithelial cells as well as genes involved in angiogenesis (e.g. *CAV1*, *EDG2*, *PDGFRL* and *CXCL12*) and extracellular matrix proteins like *SPARC* and *SPARCL1*, *FBLN1*, *RECK*.

The 95 breast cancer samples were sorted according to the three major variables (stem cell markers, ER-status and proliferation) and stratified into six groups as shown in Fig. 1. The samples in both the groups 2 and 3 were all positive for ER, negative for stem cell markers and displayed high expression of Ki67 and cell cycle markers. However, the two groups differed in their expression of the second cluster of proliferation-associated genes which are inversely correlated to Ki67.

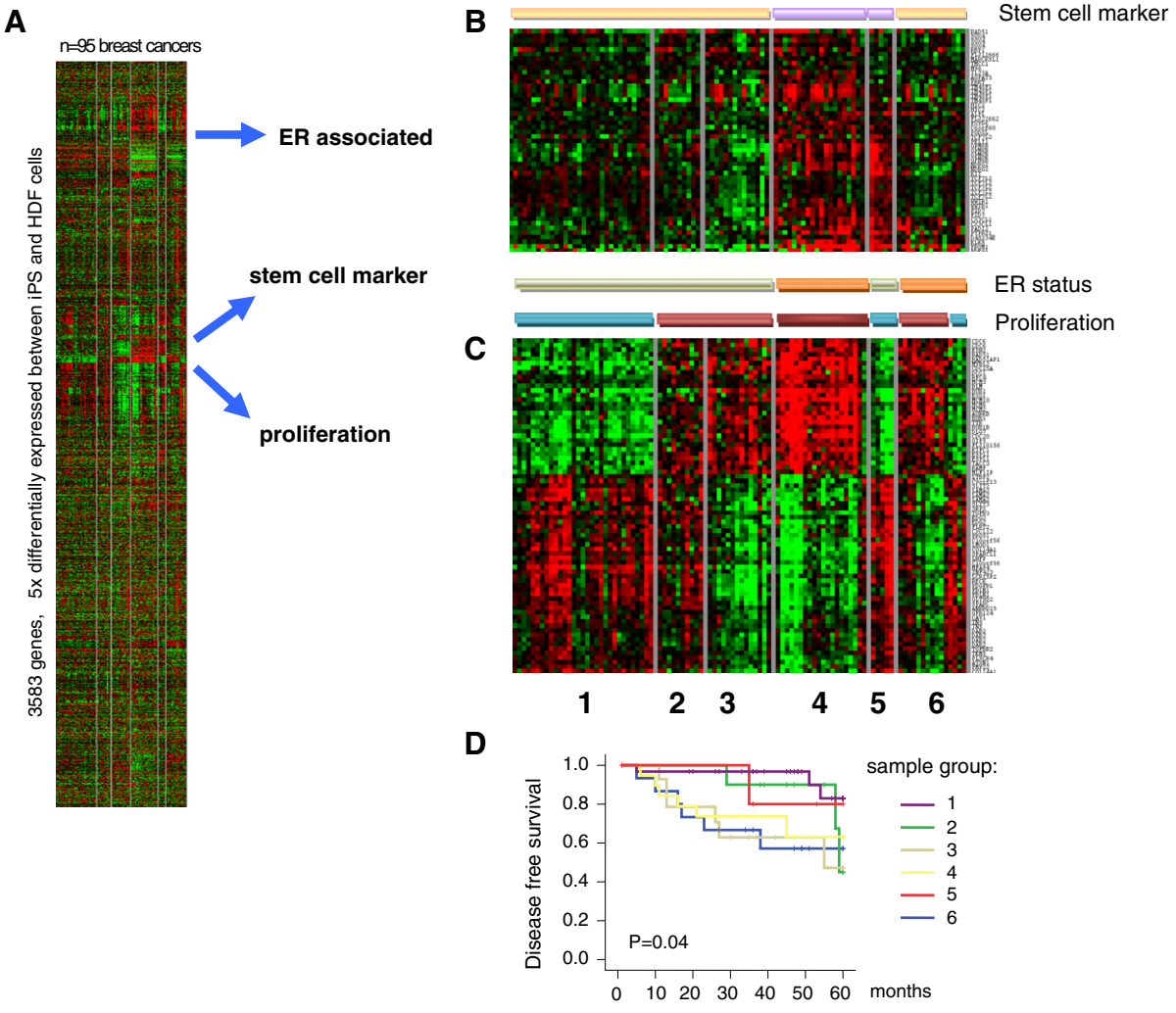

Fig. 1 – Genes showing at least fivefold differences between iPS and HDF cells ($n = 3583$; Takahashi and colleagues 2007)[1] were clustered based on their expression amongst 95 breast cancer samples (A). Samples were grouped according to the principal components represented by stem cell markers (B; violet bar above the expression matrix), the ER-status of the tumour (ER positive: green bar, ER negative: orange bar) and a cluster of genes correlated with proliferation (C; red bar: high proliferation, blue bar: low proliferation). Kaplan Meier graphs of the disease-free survival of six distinct classes of tumours are given in D.

Table 1 – Clinical parameters of patients in the different tumour subgroups from molecular analysis

			Tumour classes in Fig. 1												P-value
		Total *n*	1		2		3		4		5		6		
			n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	
Number of samples		95	31	32.6	10	10.5	14	14.7	19	20.0	6	6.3	15	15.8	
Age	>50	42	10	23.8	3	7.1	7	16.7	12	28.6	2	4.8	8	19.0	
	⩽50	53	21	39.6	7	13.2	7	13.2	7	13.2	4	7.5	7	13.2	n.s.
Lymph node status	LNN	55	17	30.9	4	7.3	7	12.7	15	27.3	4	7.3	8	14.5	n.s.
	N1	38	13	34.2	6	15.8	7	18.4	3	7.9	2	5.3	7	18.4	
Tumour size	⩽2 cm	49	17	34.7	6	12.2	5	10.2	8	16.3	3	6.1	10	20.4	
	>2 cm	46	14	30.4	4	8.7	9	19.6	11	23.9	3	6.5	5	10.9	n.s.
Histological grading	G3	41	2	4.9	2	4.9	6	14.6	18	43.9	2	4.9	11	26.8	
	G1/G2	54	29	53.7	8	14.8	8	14.8	1	1.9	4	7.4	4	7.4	<0.001
ER-status	Positive	61	31	50.8	10	16.4	14	23.0	0	0	5	8.2	1	1.6	
	Negative	34	0	0	0	0	0	.0	19	55.9	1	2.9	14	41.2	<0.001
Her2 status	Positive	18	0	0	2	11.1	3	16.7	0	.0	1	5.6	12	66.7	
	Negative	77	31	40.3	8	10.4	11	14.3	19	24.7	5	6.5	3	3.9	<0.001
Adjuvant treatment	AC	34	10	29.4	3	8.8	7	20.6	8	23.5	2	5.9	4	11.8	
	CMF	61	21	34.4	7	11.5	7	11.5	11	18.0	4	6.6	11	18.0	n.s.

Tumours in group 2 display high expression of the genes from this cluster, a characteristic which was observed amongst all other subgroups only in the low proliferating tumours.

Clinical parameters of the case series under investigation and their correlation with the different subgroups from molecular analysis are presented in Table 1. All patients in this series were treated with cytotoxic therapy. No significant differences were observed amongst the molecular subgroups regarding the two treatment schemes used (CMF and anthracycline) as well as patients' age, lymph node status and tumour size. In contrast, highly significant differences between the subgroups were found for ER and Her2 status as well as histological grading. Whilst both groups 2 and 3 were characterised by high expression of cell cycle genes, a trend for histological grade 3 tumours was observed (2 of 10 in group 2 versus 6 of 14 in group 3, $P = 0.39$).

Our analysis reveals that the two classes of breast cancers with a strong expression of stem cell markers (groups 4 and 5 in Fig. 1B) are observable, which differ in ER-status and proliferation (Fig. 1C). One class is characterised by a negative ER-status and a strong expression of proliferation markers. In contrast the second class, which is ER positive, exhibits low proliferation. In this regard, it is important to note that several authors have postulated that both ER positive and ER negative mammary stem/progenitor cell populations exist.[10–12] The identification of two breast cancer subgroups with stem cell-like features differing in their ER-status supports this hypothesis.

The prognosis of patients with tumours from the different groups is presented in Fig. 1D. Median follow-up of the cohort was 42 months (IQR 27–58 months). Albeit the sample groups are relatively small, clear differences in prognosis between the different subgroups were observed. A worse prognosis was observed for the two ER negative samples groups independent of the expression of stem cell markers (groups 4 and 6, respectively). However, samples from group 3 which contains ER positive tumours displayed a similar poor prognosis. This group was characterised by high proliferation. Interestingly, tumours in group 2 which also display high expression of cell cycle genes seem to have a better prognosis at least in the first years of follow-up.

3. Conclusion

The comparison of genes differentially expressed in HDF and iPS is confounded by the fact that many genes which play a crucial role in mammary epithelial cells are not expressed in HDF or iPS. Moreover, retroviral transfection of HDF could result in unspecific gene expression patterns. However, our analysis demonstrates that genes differentially expressed between these cell types allow a meaningful classification of different breast cancer subtypes. Furthermore, these data suggest that a genetic alteration of normal mammary epithelial stem or progenitor cells could lead to a maturation arrest of undifferentiated cells, resulting in a bulk tumour with phenotypic features of its cell of origin which influences prognosis and possibly also response to therapy.

Conflict of interest statement

None declared.

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