Ethnicity influences breast cancer stem cells' drug resistance

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Ethnicity influences Breast Cancer Stem Cells Drug resistance

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Up to 70% of Breast Cancer (BC) patients relapse within 5 years. Al-Hajj et al. reported that a subpopulation of cancer cells, Breast Cancer Stem Cells (BCSCs), has an inherent ability to resist drugs and cause relapse [1]. The biology of BC have been shown to be different in patients from different ethnic populations [2]. This racial variation is specifically evident in BCSCs resistance to chemotherapy. In Vietnam, it was shown that CD44 is the main player in resistance of BCSCs to chemotherapy [3], whereas in Japan, ALDH1 was proven to have a strong association with resistance to drugs [4]. In this study, we tested the hypothesis that BCSCs are quantitatively different between European and African ethnic groups and they use different mechanisms to resist therapy.

Twenty three BC surgical specimens were collected (see compliance with ethical standards section). 17 patients were Egyptians and 6 were English. Patients were clustered into untreated (therapy naïve) and treated (patients who received neoadjuvant therapy or their tumour cells were incubated in vitro in media containing 1µm Doxorubicin for 16 hours). Egyptian patients were 11 untreated and 6 treated whereas English patients were 3 untreated and 3 treated. All patients had the same tumour histological type (Invasive ductal carcinoma) and were estrogen receptor positive (ER+).

Anoikis resistant cells (as a measure to BCSCs) were isolated as described by Shaw *et al*. [5]. Egyptian patients had nearly 2-fold more BCSC numbers compared to English patients (32%±12.8 vs. 18%±4.3, p=0.13) (Fig.1). This is the first study to compare BCSCs numbers between different ethnic groups. Our findings agree with a study found that African Americans colorectal cancer patients had 60% more CSCs compared to their Caucasian counterpart [6]. The relatively high numbers of BCSCs in Egyptian patients may explain the poor prognosis of BC in Africa compared to well-developed countries [7].

We then investigated the expression of 46 genes known to have roles in drug resistance (Supplementary Table 1) using StellARray qPCR Arrays. We found that all genes showed significantly higher expression in BCSCs from the untreated Egyptian samples compared to those from the English group (Supplementary Table 2). Our data show that it is not only BCSC content which differs across populations, but also their drug resistance mechanisms are different. This is the first study to compare

gene expression of BCSCs between different ethnic groups. However, others reported that biology of BC is different in different ethnic groups, yet all these studies were on the level of bulk tumour cells.

Moreover, in the Egyptian group, *NOTCH4* was the only gene showing a significant increase in its expression in treated patients compared to untreated patients (Table 1). In contrast, in the English group, *NOTCH1* was the only gene showed a significant increase in its expression (Table 2). Previously we found that NOTCH4 knockdown caused greater reduction in CSC activity than NOTCH1 in the English patients [8] and NOTCH4 receptor activation mediates BCSC activity as a response to therapy [9]. The contradiction between the current study and our previous studies may be due to posttranscriptional and or posttranslational modifications of NOTCH signaling. Indeed the differential expression we detected in this study in NOTCH members among ethnic groups is on the level of mRNA expression, whereas our previous studies measured protein levels of expression and activity of NOTCH members. Our results recommend specific targeting for *NOTCH1* and *NOTCH4* in the English and Egyptian patients, respectively.

In conclusion, we were the first to show that BCSCs are qualitatively and quantitatively different in different ethnic groups which may explain the racial variation in BC behaviour and biology. This is a pilot study with a limited number of patients and therefore these findings would have to be further validated on a larger cohort of patients.

Compliance with Ethical Standards:

English samples were collected via Manchester Cancer Research Centre Biobank which has been ethically approved by the South Manchester Research Ethics Committee (Ref: 07/H1003/161 + 5). Collection of Egyptian samples has been ethically approved by the Research Ethics Committee, Faculty of Medicine, University of Tanta (Code: 3012/01/15).

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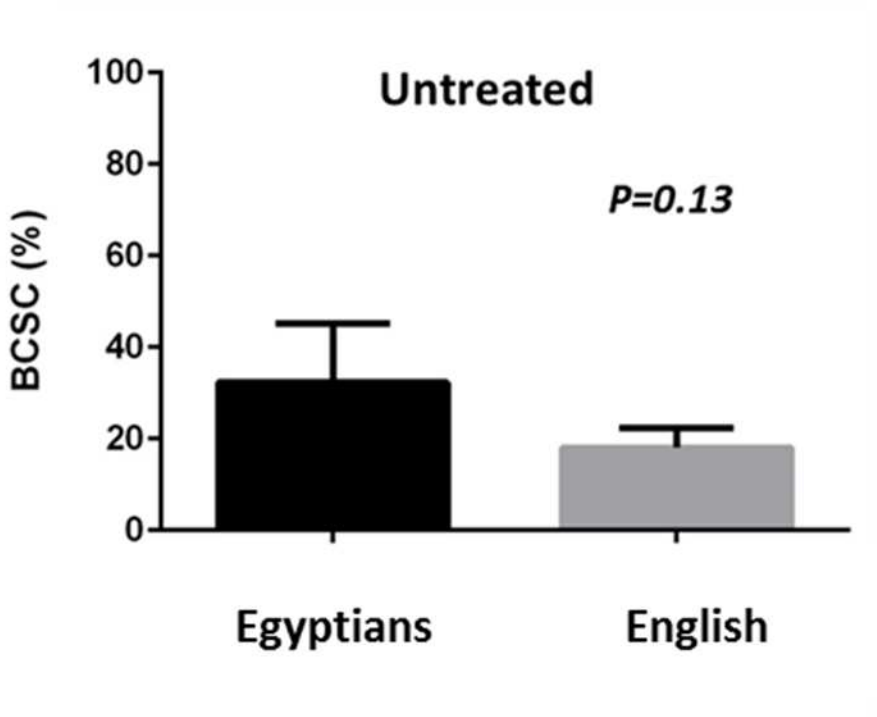

Fig 1: Percentage of anoikis resistant cells in un-treated Egyptian and English patients.

Table 1: Genes showed significant difference in their expression between BCSCs from treated and un-treated Egyptian patients

Down-regulated genes			Up-regulated genes		
Gene	**Fold Decrease**	**Function**	**Gene**	**Fold Increase**	**Function**
BMI1	1.5130362	Proliferation	NOTCH4	3.50224	CSC Signaling
CDK2	1.1117699				
CDKN1A	0.767426				
VEGFA	1.12864667				
CCNB1	1.1498215				
MMP9	0.852149				
HIF1A	1.6078917	Transcription			
NFKB1	1.024524				
NANOG	1.160355				
STAT1	1.168615				
SHH	0.798564				
CD44	1.330462	CSC Signaling			
JAG1	1.0351375				
MET	1.93902847	Metastasis			
DNMT1	4.363427	DNA methylation			
DNMT3B	0.834107				
BCL2	1.273736	apoptosis			

Fold change values are displayed with respect to the treated patients. *p* value ≤0.05 was considered significant.

Table 2: Genes showed significant difference in their expression between BCSCs from treated and un-treated English patients

Down-regulated			Up-regulated		
Gene	**Fold Decrease**	**Function**	**Gene**	**Fold Increase**	**Function**
GLI1	0.849724	GLI family zinc finger 1	NOTCH1	8.33958	CSC signaling
IL10	0.978846	Inflammation			

Fold change values are displayed with respect to the treated patients. *p* value ≤0.05 was considered significant.