The Characterisation of Hyaluronan-Related Enzymes in Breast Cancer Cell Subpopulations

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METHODOLOGY

The ZR-75-1 (HAS2^{low}) breast cancer cell line was stably transfected with HAS2 and the MDA-MB-231 (HAS2^{high}) breast cancer cell line was stably transfected with anti-sense HAS2. The parental and transfected cell lines were quantitatively characterised for gene and protein expression of HAS1-3, HYAL1-3, CD44s, CD44v6, aldehyde dehydrogenase (ALDH) and CD24 . The HAS2-ZR-75-1 cell line had a 100-fold increase in HAS2 expression while the MDA-MB-231-HAS2 knockdown cell line had a 100-fold decrease in HAS2 expression. HAS2 over-expressing and anti-sense breast cancer cell lines were separated into putative CSC (CD44$^+$/CD24$^-$) and non-CSC subpopulations (CD44$^-$) using FACS (**Figure 1**). Cell subpopulations were characterised for cell-growth kinetics, HA glycocalyx formation, chemotherapy resistance and the expression of HA metabolic enzymes (HAS and Hyal) using qRT-PCR and immunohistochemistry.

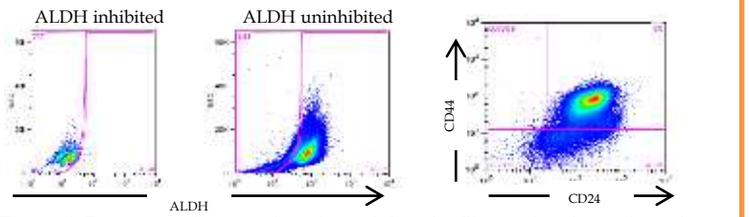

Figure 1: Representative pseudo-coloured dot plot illustrating the gating scheme implemented in the isolation of breast cancer cell subpopulations via FACS.

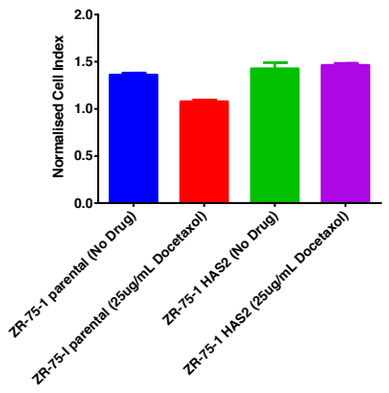

Figure 2: Increased HAS2 expression confers chemotherapy resistance *in vitro*.
These data represent treatment with Docetaxol; similar data obtained with Irinotecan and Doxorubicin treatment

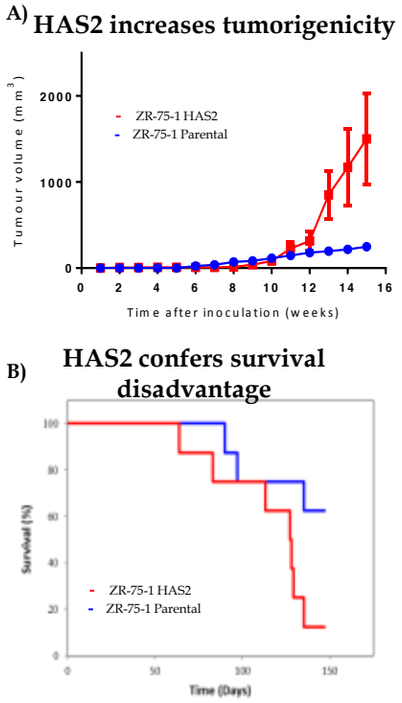

Figure 3: HAS2 promotes aggressive tumor growth *in vivo*

REFERENCES
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Csoka, A.B., *et al.*, (2001) *Matrix Biology* **20** 499-508.

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SUMMARY OF KEY FINDINGS

1. Increased HAS2 expression results in:

i) Chemotherapy resistance *in vitro:* Increased HAS2 expression conferred an inherent resistance to a selection of common chemotherapeutics including Docetaxol (**Figure 2**), Doxorubicin and Irinotecan

ii) Increased tumorigenicity and more aggressive proliferation *in vivo* where increased HAS2 expression resulted in 3-fold increase in the rate of tumor initiation and growth (**Figure 3A**). Promotion of tumor establishment and growth by HAS2^{high} cells translated into a shorter survival period characterised by rapid disease progression where only 12.5% of the population remained at experimental endpoint (Day 147) compared to 62.5% survival in HAS2^{low} tumor bearing mice (**Figure 3B**).

iii) Increased migration and metastasis *in vitro* and *in vivo* suggesting enhanced invasive properties. *In vitro*, HAS2 expression increased migration by 50% when compared to the HAS2^{low} non-transfected cell line (**Figure 4A**). *In vivo*, HAS2 expression conferred a survival disadvantage where all mice with HAS2^{high} tumor inoculants were dead at Day 159 versus 60% survival in mice with HAS2^{low} tumors at experimental endpoint (Day 225) (**Figure 4B**).

2. Altered HAS2 expression results in the reciprocal augmentation of the putative CSC subpopulation (Figure 5).

3. CD44^{positive} chemotherapy-resistant subpopulations express HAS2 and Hyal2 (Figure 6).

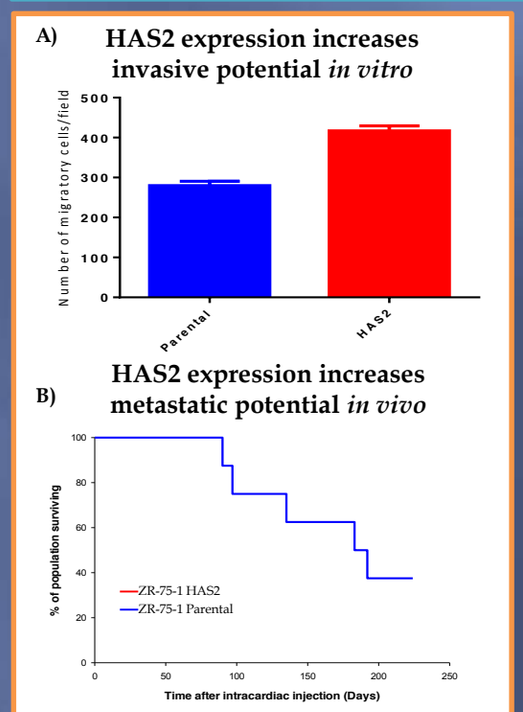

Figure 4: HAS2 enhances migration and metastasis *in vitro* and *in vivo*

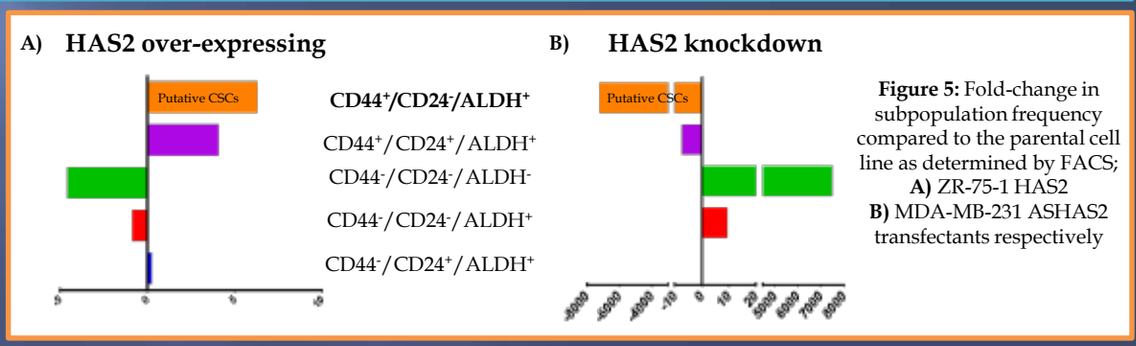

Figure 5: Fold-change in subpopulation frequency compared to the parental cell line as determined by FACS;
A) ZR-75-1 HAS2
B) MDA-MB-231 ASHAS2 transfectants respectively

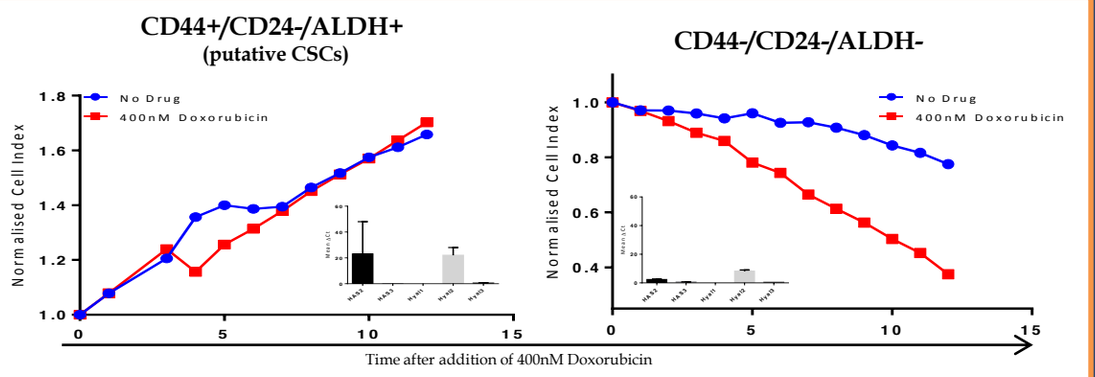

Figure 6: HAS2 and Hyal2 expression is associated with resistant subpopulations

CONCLUSIONS

These preliminary data suggest that HA metabolism is a tightly regulated process and a key component within breast cancer subpopulations that are associated with the initiation and prolongation of breast cancer tumors and treatment resistance thereby highlighting these proteins as potential targets in the eradication of breast CSCs.