

INTRODUCTION

Background: The basal cell subtype is a particularly aggressive form of breast cancer. The molecular mechanisms which underlie the aggressive course of basal cell breast cancer, as well as its corresponding tumor markers are currently under intense study. In addition, there is increasing evidence that cancer stem/progenitor cells are integral to the formation and perpetuation of breast cancer. Together with their progenitors, cancer stem cells may determine the therapeutic outcome and the clinical course of cancer (Figure 1). The first isoform of aldehyde dehydrogenase (ALDH1) has recently been reported to be a marker of breast stem/progenitor cells (Figures 2 and 3). Thus, we undertook this study to determine whether the immunohistochemical detection of ALDH1 in fixed breast cancer tissues correlates with selected basal and luminal cell surface markers.

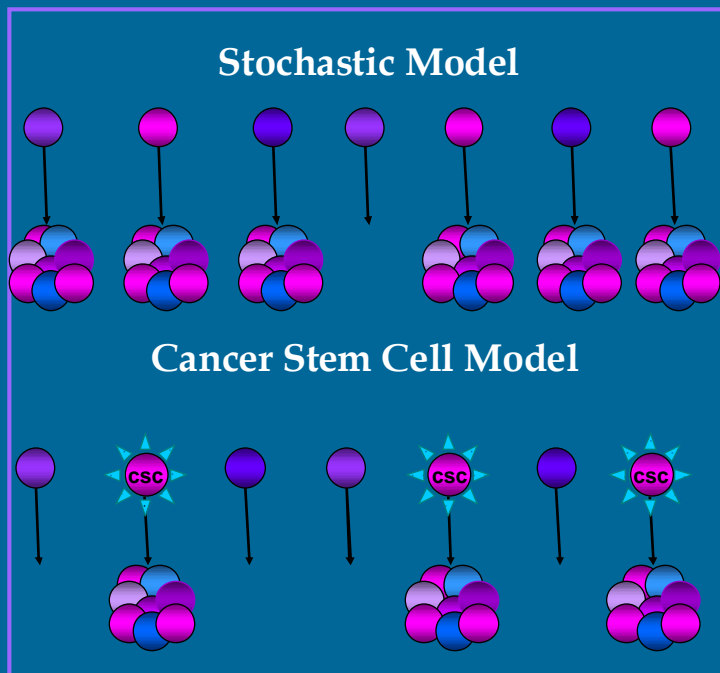


FIGURE 1: A comparison of the stochastic model where most cancer cells can proliferate extensively to give rise to new tumors with the cancer stem cell model where only a few cancer stem cells (CSC) can give rise to new tumors.

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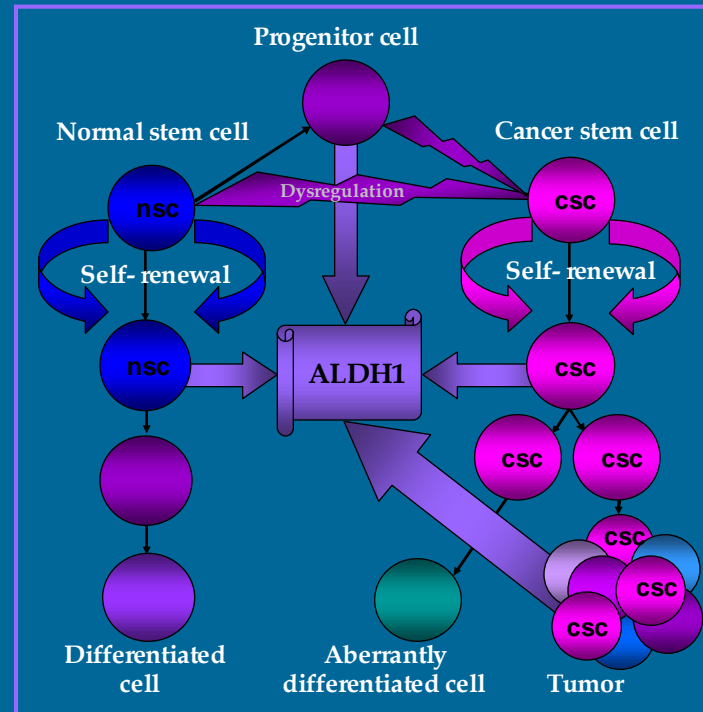


Figure 2: ALDH1 is a marker of stem/progenitor cells of the normal human breast and breast carcinomas. Cancer stem cells (CSC)s are tumor-initiating cells which may arise from the dysregulation of normal stem cells (NSCs) or mutated progenitor cells. Stem cells self-renew and differentiate. NSCs divide asymmetrically to produce a NSC and a differentiated cell. In contrast, CSCs divide symmetrically, giving rise to two CSCs and may result in clonal expansion. CSCs may also give rise to aberrantly differentiated cells. ALDH1 is a marker of stem/progenitor cells of the normal human breast and high ALDH1 levels identify the tumorigenic cell fraction of breast carcinomas.

MATERIALS & METHODS

Methods: Formalin-fixed, paraffin-embedded specimens were studied in triplicate using tissue microarrays from 57 breast cancer patients. In addition to ALDH1 staining, each of the following markers were studied using immunohistochemistry (IHC) in each sample: ER, PR, HER-2, the basal cell markers EGFR/HER1, CK5, CK14, CK17, SMA, and the luminal cell markers CK19, and EMA.

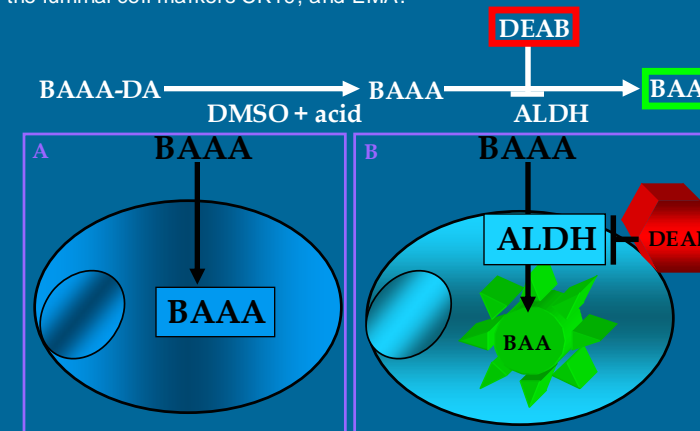


Figure 3: The ALDEFLUOR® assay. The ALDEFLUOR® assay is illustrated in A) ALDH-negative cells and B) ALDH-positive cells. In the presence of dimethylsulfoxide (DMSO) and acid, BAAA-DA Bodipy™-aminoacetaldehyde diethyl acetal (BAAA-DA) is converted to the ALDH substrate, Bodipy™-aminoacetaldehyde (BAAA), which diffuses freely across the plasma membrane of intact viable cells. Intracellular ALDH converts BAAA into the fluorescent product BAA (BODIPY®-aminoacetate). In the presence of ALDH, cellular fluorescence can be detected with the green fluorescence channel of a standard flow cytometer and compared with treatment using the ALDH inhibitor diethylamino-benzaldehyde (DEAB) as a negative control (<https://www.stemcell.com>).

RESULTS

Results: ALDH1 staining was performed using IHC and subsequently evaluated using the Quick score. The Quick score takes into account both the percentage of positive cells, as well as the corresponding staining intensity. Our results suggest that breast cancer samples expressing the triple negative phenotype (ER-, PR-, HER-2 negative) together with one or more basal cell markers (EGFR/HER1, CK5, CK14, CK17, SMA) are associated with an elevated Quick score for ALDH1 staining, when compared with samples lacking the expression of these markers.

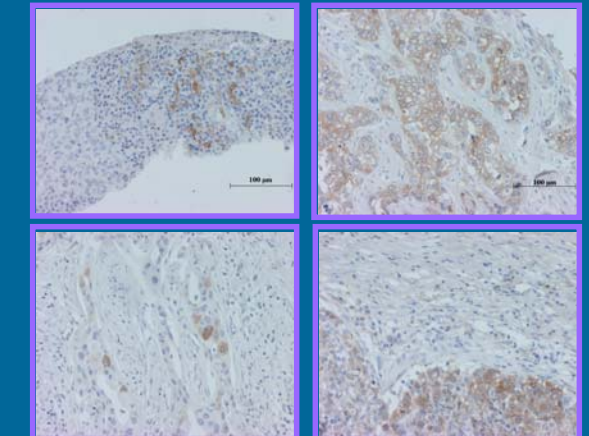


Figure 4: Examples of ALDH1-positive breast cancer cells detected with immunohistochemistry.

CONCLUSIONS

Conclusions: As it has been demonstrated that IHC detection of ALDH1 detects stem/progenitor cells in fixed breast samples, our results suggest that an elevated Quick score is associated with the enrichment of IHC basal cell markers in these samples. We are currently focusing on a larger number of breast cancer samples so that correlations can be studied as a function of therapeutic outcome and overall survival of breast cancer patients.

REFERENCES

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