Breast Cancer RESEARCH

VIEWPOINT

The 'alternative' EMT switch

Pamela Klingbeil* and Clare M Isacke

Abstract

Epithelial to mesenchymal transition (EMT) is an essential process in embryonic development and is aberrantly induced in many disease settings. Work carried out by Chonghui Cheng's laboratory addressed the involvement of alternative RNA splicing in EMT and its link to tumour progression. They describe a switch in CD44 expression from variant isoform(s) to the standard isoform and showed, for the first time, that this is required for normal epithelial cells to undergo EMT. In addition, they link expression of the CD44 standard isoform with high-grade breast cancer and to activation of the phosphoinositide 3-kinase/Akt pathway and apoptosis resistance in a mouse model of recurrent disease.

Background

Epithelial to mesenchymal transition (EMT) is characterised by the acquisition of a mesenchymal, motile phenotype and is accompanied by characteristic molecular changes, including the cadherin switch that is a hallmark of EMT. This switch in expression from junction-forming E-cadherin to the motility promoting N-cadherin is induced by transcriptional regulators such as Twist and Snail [1]. In addition, alternative splicing of mRNA precursors can also influence EMT [2].

The cell–cell and cell–matrix adhesion molecule CD44 is highly susceptible to alternative splicing as the *CD44* gene spans 20 coding exons, 10 of which are variantly expressed. Generally, expression of CD44 variant isoforms (CD44v) is common in epithelial cells, while the standard isoform (CD44s), with all variant exons excised, is expressed by haematopoietic and mesenchymal cells. Alternative splicing of CD44 is known to be deregulated during pathological processes including tumour invasion and metastasis, but evidence for a correlation of tumour behaviour with the different CD44v is sparse and can be contradictory [3]. As the last steps of tumour progression require dynamic adhesive properties from a cell, switching between an adhesive state and a motile state is fundamental for successful metastasis formation. EMT and its postulated reversion are crucial processes involved in this cellular plasticity.

Article

In a recent article, Chonghui Cheng's laboratory demonstrated a functional role for CD44 alternative splicing during EMT [4]. Several nontumorigenic epithelial cell lines were used for EMT induction with different EMT triggers. In all cases this induction was accompanied by a switch in CD44 isoform expression from CD44v to CD44s and, using a variety of approaches, was demonstrated to be essential for EMT. Importantly, the overall level of CD44 protein did not change significantly during this process. Isoform specificity of this effect was confirmed using RNA interference and rescue experiments, and clearly demonstrated that expression of CD44s, and not loss of CD44v, was required for cells to undergo EMT in these systems. Mechanistically, strong data were provided highlighting the role of the epithelial-specific splicing factor ESRP1. Expression of ESRP1 was downregulated during EMT, leading to reduced CD44v expression and increased CD44s levels. Furthermore, CD44s expression, but not CD44v expression, was functionally linked to Akt activation, upregulation of anti-apoptotic molecules and apoptosis resistance.

These studies were then extended to a mouse model of breast cancer recurrence and a small cohort of human breast tumour samples, which revealed a correlation of CD44s expression with recurrent and high-grade breast cancer, respectively, as well as with a mesenchymal phenotype.

Viewpoint

The important finding of this article is the functional role of CD44s expression during EMT. CD44 isoform switching and the involvement of the splice factor ESRP1 have been described previously [5-7], but the data presented by Brown and colleagues significantly extend these studies by clearly demonstrating a causal role for CD44 isoform switching in EMT and suggesting CD44s as the main substrate for ESRP1 during EMT [4]. The

*Correspondence: Pamela.Klingbeil@icr.ac.uk
Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK

authors provide clues as to how CD44s, but not CD44v, promotes EMT by demonstrating that only CD44s expression is associated with Akt activation in this system. As all CD44 isoforms share a common cytoplasmic domain, these data suggest that isoform-specific signalling during EMT should involve differential interactions of the CD44 extracellular domain. CD44 can interact with the microenvironment in multiple ways – for example, via acting as a co-receptor for transmembrane receptors like c-Met or by mediating interactions with the extracellular matrix via binding to its principle ligand hyaluronan [8], which has been previously implicated in EMT promotion. In MDCK and MCF-10A cells, hyaluronan synthase-2 expression induces EMT and activates the phosphoinositide 3-kinase/Akt pathway [9]. Further, in a model of fibrosis-associated EMT, TNFα was shown to induce transcriptional upregulation of CD44 and its subsequent pericellular interaction with hyaluronan, leading to cell–cell dissociation and cellular motility [10]. In this respect, it would be of interest to determine the potential role played by TNFα and other external factors in ESRP1 regulation.

Alternative splicing is known to be deregulated during tumour progression [11], and CD44v expression has been repeatedly linked to metastasis formation. Brown and colleagues linked switching to the CD44s isoform with tumour recurrence in the HER2/*Neu* mouse model [4]. Although the data presented are of interest, in our view the chosen mouse model has limitations for the study of breast cancer progression as it does not recapitulate well the human situation. Recurrent and secondary breast cancers usually have a very similar phenotype to the primary tumour, arguing for EMT during invasion and metastasis to be transient in nature. In the small cohort of human breast tumours examined, CD44s expression correlated with a high-grade N-cadherin-positive phenotype. Future studies with larger patient cohorts will be required to assess the diagnostic value of this observation and to determine whether Akt activation is indeed linked to CD44s expression in tumour samples.

Both EMT and high CD44 expression have been ascribed to cells with tumour-initiating properties, so-called cancer stem cells, in breast cancer and other carcinomas [8,12]. Importantly, Brown and colleagues' article addresses a potential functional role for CD44s in cancer stem cells by promoting EMT and induction of apoptosis resistance [4]. As the overall protein level of CD44 did not change during EMT, these data will encourage researchers to examine CD44 isoform specificity rather than focusing on high expression levels.

Together, these findings argue for the epithelial phenotype to be actively maintained by attenuating CD44s expression at the expense of producing alternatively spliced variants. The demonstration that overexpression of ESRP1 was able to block the splicing switch and maintain an epithelial phenotype raises the question of whether upregulating ESRP1 levels in mesenchymal cells could restore an epithelial phenotype. This hypothesis has the intriguing therapeutic possibility of normalising a highly potent metastatic cell by reversing EMT.

Abbreviations

EMT, epithelial to mesenchymal transition; CD44s, CD44 standard isoform; CD44v, CD44 variant isoforms; TNF, tumour necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Published: 16 August 2011

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doi:10.1186/bcr2915
Cite this article as: Klingbeil P, Isacke CM: **The 'alternative' EMT switch.** *Breast Cancer Research* 2011, **13:**313.