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Divide and conquer: the genetic basis of molecular subclassification of breast cancer

Vessela N. Kristensen

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Since the luminal B tumours are associated with poor recurrence-free and disease-specific survivals in all adjuvant systemic treatment categories including hormone therapy, the identification of specific signalling pathways driving luminal B biology is paramount to improve treatment. Sircoulomb et al. and Holland et al. have independently identified the *ZNF703* gene, located in chromosomal region 8p12, as preferentially amplified in luminal B tumours.

The human genome project and the development of high throughput parallel microarray analysis of gene expression revealed the existence of distinct molecular profiles of cancer and suddenly cancers were divided into molecular subclasses/sub-diseases previously unknown to classical pathology. In their groundbreaking paper, Perou et al. studied the patterns of gene expression in two consecutive samples of locally advanced breast cancer (Perou et al, 2000). By means of unsupervised hierarchical clustering, they showed that it was possible to differentiate genomic signatures in breast cancer based on the differential expression of ~534 genes, and several other classifying signatures have been introduced thereafter (Nielsen et al, 2010). These investigations provided the basis of molecular taxonomy and identified five different molecular subtypes of breast cancer. Three of these classes are characterized as having low to absent expression of estrogen receptor (ER) and other specific transcription factors when compared to the other subtypes. These three are referred to as (i) basal-like subtype, characterized by high expression of keratins 5 and 17, laminin and fatty acid binding proteins; genes that are often more expressed in the basal cell of normal breast ascini; (ii) the ERBB2+ subtype, characterized by higher expression of the epidermal growth factor (EGF) receptor family member and other genes associated with amplification of the ERBB2 locus at 17q22.24, which includes the growth factor receptor adaptor protein GRB7; and (iii) the normal-like subtype, characterized by expression of a large number of genes normally expressed in other tissues of non-epithelial origin and higher expression of genes more often associated with basal epithelial cells than luminal epithelial cells. The last two remaining molecular phenotypes are breast tumour subtypes referred to as luminal A and luminal B. These two groups characteristically have the highest expression of ER. In addition, luminal A subtype is characterized by higher expression of the transcription factors GATA3, hepatocyte nuclear factor 3α, estrogen-inducible secreted factor trefoil factor 3 (TFF3), and estrogen-induced solute carrier SLC39A6/LIV-1. Luminal B is characterized by lower expression of luminal type genes and high expression of proliferation markers. These sets of genes have been repeatedly shown to reliably segregate breast carcinomas derived from independent data sets and samples into these five specific molecular subtypes (Sørlie et al, 2001, 2003). In 2002, van't Veer et al. reported a gene-expression profile that was associated with prognosis in patients in terms of the likelihood of distant metastases within five years (van't Veer et al, 2002). These and other seminal papers opened the route to the development of numerous computational methods aimed at identifying trends and patterns of gene expression specific to different tumours and subtypes and have led to the discovery of several genetic patterns or molecular signatures that aid in distinguishing biologically relevant aspects of tumour behaviour, function and identity.

Ten years later, the breast cancer clinical community debates over the usefulness and superiority of the molecular prognostic potential of these molecular classes. Basic researchers have embarked on a long and convoluted journey to unravel what exactly this molecular classification means in terms of both deregulated genes and biological pathways, as well as the admixture of cells it represents. The multiple mechanisms of this deregulation are reflected in the fact that this molecular classification can be recapitulated, to a large extent and further refined by studies of the promoter composition of the genes that condition it (Tongbai et al, 2008), the copy number

Institute for Cancer Research, Oslo, Norway.
Corresponding author: Tel: +49 6221 8891 310; Fax: +49 6221 8891 240;
E-mail: v.n.kristensen@medisin.uio.no
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aberrations that underlie it (arrayCGH and SNP-CGH) (Pollack et al, 1999; Russnes et al, 2010; Van Loo et al, 2010), and the DNA methylation status (Holm et al, 2010; Roenneberg et al, 2011) as well as using whole genome miRNA expression studies (Blenkiron et al, 2007; Enerly et al, 2011) and analyses of TP53 mutation status of the tumours (Langerød et al, 2007).

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In this issue of EMBO Molecular Medicine, two back to back publications (Holland et al, 2011; Sircoulomb et al, 2011) provide further evidence that the heterogeneity of breast carcinomas can be explained by distinct somatic alterations at the DNA level and that these alterations involve genes with specific roles in downstream deregulation. These authors focus on a particular subtype of breast cancer, the luminal B. This subtype is especially interesting for its diversity, which makes it more vulnerable than others to the choice of classification method and is hence difficult to stably identify. Luminal B tumours have lower expression levels of estrogen receptor (ER)/progesterone receptor (PR) and related genes, higher proliferative rates and are often of higher grade. In addition, some tumours defined as luminal B by expression array, are human epidermal growth factor receptor (HER)2 positive. Since the luminal B tumours are associated with poor recurrence-free and disease-specific survivals in all adjuvant systemic treatment categories including hormone therapy, the identification of specific signalling pathways driving luminal B biology is paramount to improve treatment. Sircoulomb et al. and Holland et al have independently identified the *ZNF703* gene, located in chromosomal region 8p12, as preferentially amplified in luminal B tumours (Holland et al, 2011; Sircoulomb et al, 2011). These authors first identified somatic DNA copy number alterations that were prevalent in the luminal B subtype. In the case of Sircoulomb et al. recurrent high-level amplifications (8p12, 8q22, 11q13, 17q24, 20q13) were observed first in 41 luminal B and 59 luminal A cases from a series of 266 and then in 1172 breast cancers from 11 different published datasets which included 561 luminal tumours (Luminal A and B, Sircoulomb et al, 2011). Holland et al. based their observations initially on 171 breast tumours and then expanded to a total of 1001 primary breast carcinomas (Holland et al, 2011). Both groups concluded that the *ZNF703* gene is a likely oncogene candidate as it is the most frequently amplified and overexpressed in this region across all the populations studied and embarked on a variety of molecular functional studies to characterize its function (Fig 1). Sircoulomb et al. performed an *in silico* analysis

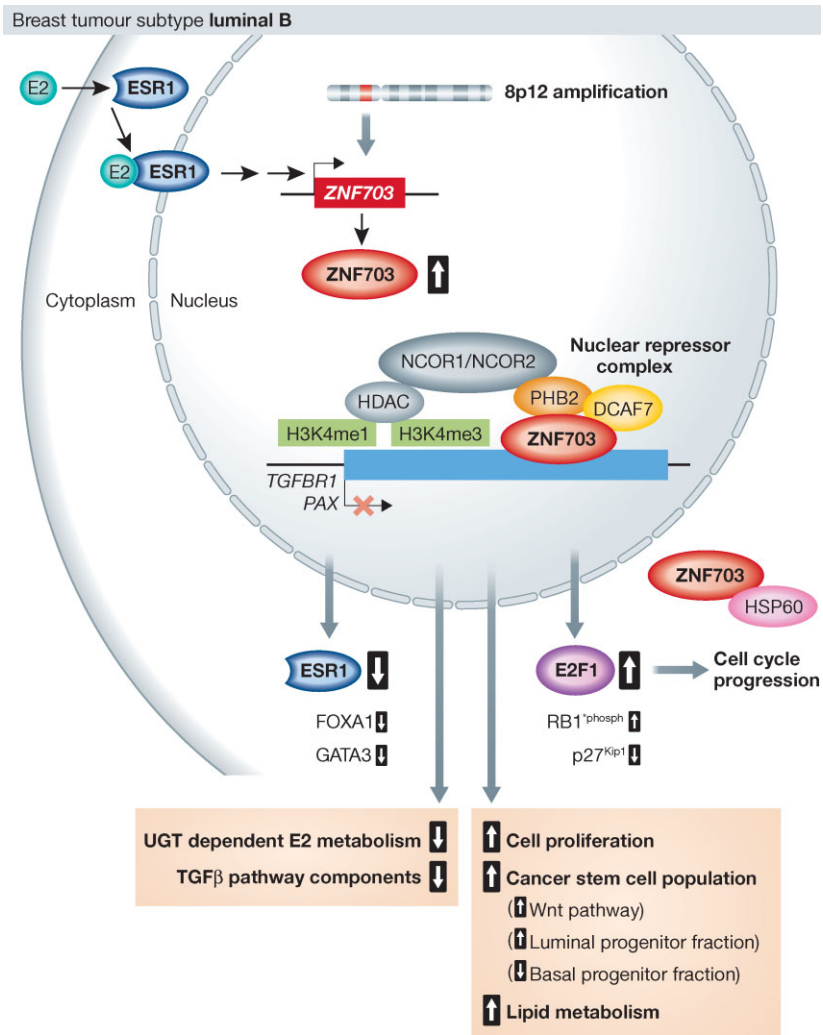

Figure 1. ZNF703 function in luminal B breast cancer. Transcription of the amplified *ZNF703* gene is activated by E2-ESR1 signalling. ZNF703 forms a nuclear repressor complex together with DCAF7, HSP60 and PHB2 (Prohibitin 2). ZNF703 was found to co-localize with HDAC1 on promoters with H3Kme1 or H3K4me3 labels suggesting mediation of active repressing of genes such as PAX2 and TGFBP2. The enhanced levels of ZNF703 lead to a variety of downstream effects like downregulation of important ER transcriptional targets and up-regulation of E2F1 with following increase in RB phosphorylation and decrease of P27kip1with subsequent cell cycle progression into proliferation depending on the subtype of mammary cell.

of transcription factor binding sites in the luminal B expressed genes and, using co-expressed reporter vectors, identified *ZNF703* as regulating E2F1 and ER transcriptional activity (Sircoulomb et al, 2011). They showed that *ZNF703* transcription and protein production are induced by addition of 17β-estradiol (E2) in a time-dependent manner in MDA-MB-134 and HCC1500 breast cancer cell lines. When overexpressed, ZNF703 induced cell proliferation as well as the expression of some 160 genes, many of which related to WNT (such as LEF1, TCF12, WNT4) or NOTCH (such as ASCL1, HEY2, TLE4) signalling pathways. When stimulated by E2, MCF7 GFP-ZNF703 cells presented a significant increase in primary *in vitro* tumorsphere formation. Furthermore, Holland et al examined the presence of mammary progenitor cells within adult human breast tissue and after separation of the luminal and basal enriched fraction, showed that the infection with lenti-*ZNF703* preferentially caused an increase in colony counts for the luminal progenitor enriched cell fraction and a decrease for the basal one (Holland et al, 2011). In addition, they showed that ZNF703 overexpression led to transformation, strongly suggesting that it represents a classical oncogene. GFP-ZNF703 was mainly found localized in the nuclear matrix in subcellular dot-like structures and SDS-PAGE analysis revealed three potential ZNF703-interacting proteins. All peptides were identified by MS analysis as three different proteins: DCAF7, HSP60 and PHB2 (Prohibitin 2), suggesting that ZNF703 is a cofactor of a nuclear co-repressor complex, modulating ER transcriptional activity but also controlling breast cancer stem cell differentiation by decreasing ER and increasing E2F1 transcriptional activities. A subsequent increase of RB1 phosphorylation and a decrease of P27kip1 protein expression were observed in MCF7 GFP-ZNF703 cells, consistent with the drive towards cell cycle progression and proliferation.

In summary, the findings in both papers may suggest that although expressed in Luminal B subtypes, the ER is decoupled from its downstream signalling by the nuclear co-repressor complex of which ZNF703 is a part of, together with HDAC1 or 2. Potentially, this finding may have a therapeutic significance as HDAC inhibitors may unblock this repressor activity thus restoring ER signalling, making the tumours again vulnerable to hormonal treatment. Whether this described pathway of molecular deregulation is specifically active in Luminal B like breast cancers remains to be seen as the evidence is still circumstantial, based on the predominant occurrence of the 8p12 amplification in this type of cancers in these datasets. There are unfortunately no good model cell lines for Luminal B cancer and one cannot exclude that increased expression of ZNF703 may occur in other subtypes of breast cancer as well through up-regulating mechanisms other than amplification. It is also still possible that additional genes on this and other 'Luminal-B specific' amplicons may play their own role, although the authors make a strong argument that this is the only gene that was found under ER regulation. In any case, these studies provide an exemplary effort to divide breast cancers based on their overall genetic characteristics and specific molecular pathways, making them more accessible for the development of targeted treatment. Yet they illuminate a very small corner of a large puzzle. Hopefully, more such studies will come in the future.

The author declares that she has no conflict of interest.

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