Cell Cycle
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kccy20

CRH-R splicing in estrogen-sensitive breast cancer

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Published online: 21 Jan 2014.

Cell Cycle 13:5, 687–688; March 1, 2014; © 2014 Landes Bioscience

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Alternative pre-mRNA splicing contributes in the acquired genomic alterations involved in the pathobiology of cancer development and progression.[1] In normal biological systems, this mechanism allows protein complexity and diversity by increasing gene coding capacity and promoting expression of several related proteins with diverse and even antagonistic functions. However, in pathological settings such as cancer, mutations in splicing regulatory elements and/or alterations in the cellular splicing machinery can change splicing patterns and result in the generation of aberrantly spliced pre-mRNAs that favor development of the malignant state.

In breast cancer, an overall upregulation of splicing factors and remarkable changes in the splicing mechanisms has been identified, characterized by exon skip and intron retention as the predominant events.[2] These result in cancer-specific splice variants for various genes with important roles in regulation of tumor cell proliferation, DNA damage, adhesion, invasion, angiogenesis and apoptosis, and response to therapy. For example, a survivin variant with pro-apoptotic properties that acts as a naturally occurring antagonist of the anti-apoptotic oncogene BIRC5 is downregulated in breast carcinoma. Increased expression of alternatively spliced cadherin-11 variants enhances breast cancer cell invasive potential. Also, MDM2, a phosphoprotein that participates in the proteasomal degradation of the tumor suppressor p53, can generate more than 40 aberrant alternatively spliced transcripts. Expression of specific MDM2 variant transcripts has been correlated with a shorter overall survival of breast cancer patients. VEGF isoforms have been shown to be prognostic for survival in patients with node-positive breast cancer, whereas CD44 novel variant isoforms are overexpressed and preferentially located in metastatic breast cancer tissues.

Modifications in the alternative splicing profile of CD44 during mammary gland tumorigenesis, are associated with dramatic changes in abundance of the arginine–serine-rich (SR) family of splicing factors,[3] known to influence alternative splicing decisions in a wide variety of genes. Our recent study[4] revealed that one of these SR nuclear proteins, SRp55, encoded by the *SRSF6* gene, is targeted and downregulated by estrogens in an estrogen-sensitive malignant mammary epithelial cell line. This strengthens emerging evidence that points toward an important role of estrogens as regulators of alternative splicing through modulation of SR factors expression: a recent study[5] in ER-positive breast cancer cells, identified altered splicing events in 154 genes under the influence of estrogens, with 40% of genes lacking intragenic ERα binding sites. The list of targets included genes important for cell apoptosis, and this event is linked to resistance to apoptosis and response to therapy. This study also suggested that the *SRSF7* factor is estrogen-inducible and may contribute to estrogen-regulated alternative splicing of genes without ERα binding sites by causing splicing as a secondary event. SRp55 has been previously implicated in the pathogenic mechanisms of breast cancer, and depletion of SRp55 levels is associated with increased resistance to DNA damage, whereas mutations in the *SRSF6* gene have been identified in breast and colorectal cancer; such mutations could transform these factors as oncoproteins or tumor suppressors, depending on their antagonistic functions on splice site selection.

SRp55 has been previously shown to control the splicing profile of several tumor-associated genes, such as *FGFR1*, *CD44*, and *KIT*; our study identified the type 1 corticotropin-releasing hormone receptor (CRH-R1), a member of the class B1 G-protein-coupled receptors (GPCR) as a novel target. In mammals, CRH-R1 mediates and controls cellular actions of CRH, the hormonal master switch of adaptive responses to stress. Estrogen-induced changes in SRp55 levels appear to alter splicing direction of the *CRHR1* gene toward exon-12 skipping and increase expression of signaling-impaired, "exon-12-less" CRH-R1 mRNA variants, thus limiting cellular responsiveness to CRH actions. This in-frame deletion of 42 bp from the CRH-R1 mRNA transcript leads to loss of 14 amino acids from the C-terminal end of TMD7.

Potentially, this is a splicing "hot spot" conserved throughout evolution, since the 42-bp exon is present in the genomic sequence of all members of the B1 GPCRs and several members of the ancestral family of adhesion GPCRs,[6] suggesting a function preserved across different members.

These findings have expanded our appreciation of the complexity of CRH signaling regulation. The biological consequences of E2-driven neutralization of CRH signaling through aberrant splicing in breast cancer cells are intriguing and might be of relevance for a wider group of GPCRs and their cognate agonists in cancer or other pathophysiological settings such as pregnancy and labor.[7] Exposure to chronic stress represents a major risk factor for the development and progression of cancer, and CRH-induced molecular

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Submitted: 10/09/2013; Accepted: 10/28/2013; Published Online: 01/21/2014
http://dx.doi.org/10.4161/cc.27858
Comment on: Lal S, et al. Sci Signal 2013; 6:ra53; PMID:23821771; http://dx.doi.org/10.1126/scisignal.2003926

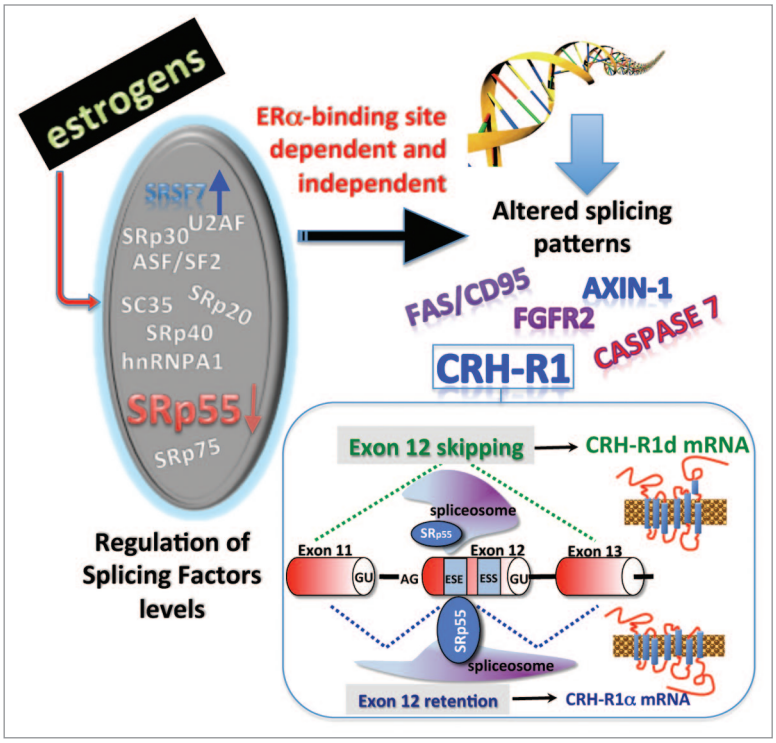

Figure 1. Estrogen-induced splicing in breast cancer cells and consequences for cell responsiveness to CRH. Estrogens are able to induce a plethora of splicing events in breast cancer cells, via regulation of expression of a distinct subset of splicing factors. This allows generation of cancer-specific splice variants for various genes with important roles in tumor cell biology. Downregulation of SRp55 alters splicing direction of the *CRHR1* gene toward exon-12 skipping and increases expression of "exon-12-less" CRH-R1 mRNA variants. These variants exhibit impaired signaling, and, therefore, this molecular event might act as a signaling checkpoint by limiting cellular responsiveness to CRH actions.

mechanisms activated in response to stressful stimuli might increase disease susceptibility and facilitate neoplastic transformation and cancer pathogenesis.[8] Although at present we can only speculate about the actions of CRH in breast cancer cells, it appears that CRH exerts complex tumor-promoting as well as anti-tumor roles in in ER-positive breast cancer cells, through activation of an intricate network of kinase-triggered signaling pathways and regulation of multiple transcriptional targets.

This emerging pattern of CRH actions thus highlights the need of further studies to elucidate this, in particular in the context of estrogen-sensitive breast cancer. Nevertheless, this novel functional link between estrogens and aberrant *CRHR1* splicing pattern provides new insights about cellular checkpoints of responses to hormonal signal transduction. (**Fig. 1**)

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