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Steroid Receptor Coactivators and Their Expression, Regulation and Functional Role in Endocrine Responsive and Resistant Breast Cancer

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1. Introduction

Breast cancer is the most frequent malignancy and one of the leading causes to cancer related deaths in women. Most human breast cancers express the estrogen receptor (ER) which belongs to the family of nuclear receptors and is a ligand-regulated transcription factor. It is well established that the natural ligand of ER, estrogen, has pro-carcinogenic and growth promoting effects in the mammary epithelium by stimulating proliferation and leaving the cells prone to mutations during cell cycle progression (Foster et al., 2001). Endocrine treatment of hormone sensitive breast cancer targets the estrogen activity in breast cancer cells by blocking the ER with a selective ER modulator (SERM) such as tamoxifen or inhibiting estrogen synthesis using aromatase inhibitors such as anastrozole or letrozole. Endocrine treatment decreases mortality, prolongs disease-free survival and can even reduce the incidence of breast cancer in women at increased risk (Cuzick et al., 2003). Approximately 70 % of women with ER positive tumors respond to endocrine therapy, but resistance do occur, either *de novo* or develop over time. The molecular mechanism involved in endocrine resistance is one of the central areas of breast cancer research.
The trancriptional activity of the ER is not only regulated by its ligands, but also by the level and activity of coregulator proteins. Nuclear receptor coactivators serve as adapters between the receptor and the trancriptional machinery. They possess diverse enzymatic acitivities such as histone acetyltransferase, histone methyltransferase, chromatin remodeling and ubiquitin-conjugation activity and are involved in every step of ER regulated transcription, from chromatin remodeling to transcriptional termination. The members of the p160 family of coactivators are some of the best studied coactivators. These steroid receptor coactivators (SRCs) are small proteins of 160 kDa with similar structural and functional properties, and include SRC-1, SRC-2/transcription intermediary factor-2 (TIF-2) and SRC-3/amplified in breast cancer 1 (AIB1). The SRCs are not only crucial to ER mediated effects in normal tissue. They have also been shown to be involved in the carcinogenic process and are

overexpressed in breast cancer. In addition, the SRCs are of relevance to the tissue-specific effects of tamoxifen and data suggest that they may be important to the cellular sensitivity to endocrine treatment. The SRCs and their expression, regulation and functional role during endocrine treatment in breast cancer *in vitro* and *in vivo* are the focus of this chapter.

2. Expression and functional role of SRCs in breast tissue

The SRCs are genetically distinct, but have structural similarities with 40 - 55 % sequence homology (Xu & Li, 2003). Although the SRCs have similar functional properties, experimental evidence indicates different physiological functions for the SRCs, which in part can be explained by tissue-specific expression levels, different affinities for the various nuclear receptors and variations in post-translational modifications (Chauchereau et al., 2003; Fenne et al., 2008; Hoang et al., 2004; Wu et al., 2004; Xu et al., 2009). In the classical activation of ER-mediated gene transcription, the ligand estradiol (E2) binds to the ER and promotes binding of the ER-E2 complex to the estrogen receptor element (ERE) of the target gene promoter. Here they recruit coregulatory proteins to a multi-subunit complex for gene transcription (Mangelsdorf et al., 1995; Pearce & Jordan, 2004). The ER has an activation function-1 (AF-1) domain in the N-terminus whereas the centrally located DNA-binding domain (DBD) is responsible for specific binding of the ER to EREs on target genes. The dimerization domain contains the ligand binding domain (LBD) and the AF-2. The AF-1 contributes to the constitutive estrogen-independent activation by the receptor and is separated by a hinge region from the AF-2. The SRCs have a basic helix-loop-helix (bHLH) domain, a Per-Arnt-Sim (PAS) domain and a nuclear hormone interaction domain (NID) including three LXXLL-sequences where L is leucine and X any amino acid (Fig. 1).

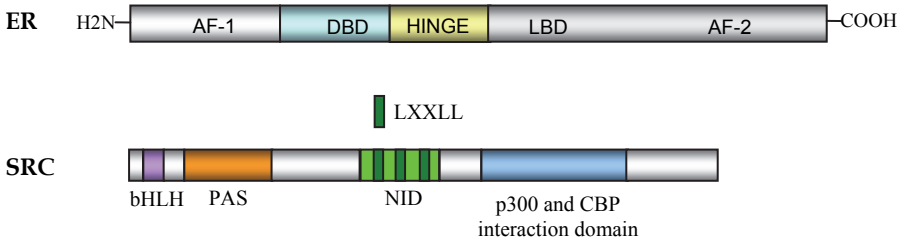

Fig. 1. Schematic presentation of the functional domains of the ER and the SRCs.

Upon ligand binding, the helix 12 of the ER is positioned across the LBD, and together with helices 3-5 form a hydrophobic groove where the LXXLL motifs of coactivators can bind (Heery et al., 1997). The coactivators in the multi-subunit protein complex alter the chromatin structure and facilitate recruitment of the RNA polymerase II and the basal transcriptional machinery in a programmed cyclic manner to initiate transcription of the target gene (Metivier et al., 2003).

The SRC family members are widely expressed and are detected in tissues such as placenta, testis, pancreas, lung, kidney, liver and brain (Xu & O'Malley, 2002; Xu & Li, 2003). The expression levels of the three SRC proteins in normal human breast epithelial cells are variable, but usually very low (Xu et al., 2009). SRCs are known to be overexpressed in

several types of human cancers, including breast cancer, where their overexpression is due to enhanced cellular amplification of their genes, and/or by a decrease in the intracellular degradation process of the coactivators (Xu et al., 2009). The SRCs are known to contribute to proliferation and development of breast cancers by mechanisms such as stimulation of the G1 to S phase transition during cell cycle by regulation of expression of mitogenic genes such as *c-MYC* and *cyclin D1* (Dubik & Shiu, 1992; Sabbah et al., 1999).

2.1 SRC-1

SRC-1 was the first nuclear receptor coactivator to be cloned and identified (Onate et al., 1995). The gene is located on the short arm of chromosome 2 at position 2p23 (Carapeti et al., 1998). SRC-1$^{-/-}$ mice show partial steroid hormone resistance and hepatic insufficiency (Xu et al., 1998; Louet et al., 2010). Noteworthy, ovariectomized female SRC-1$^{-/-}$ mice have decreased uterine growth and reduced mammary gland ductal side branching and alveolar formation in response to estrogen compared with wild-type mice, indicating a role of SRC-1 in estrogen-regulated breast development (Xu et al., 1998; Xu & Li, 2003). We found higher SRC-1 mRNA levels by real-time RT-PCR in human breast cancer samples compared to normal breast tissue (Haugan Moi et al., 2010), and the same has been observed at the protein level (Hudelist et al., 2003; Fleming et al., 2004b; Myers et al., 2004). SRC-1 protein expression in breast cancer has been reported to associate positively with human epidermal growth factor receptor 2/neu (HER-2/neu) and negatively with ERβ (Fleming et al., 2004b). *In vitro* studies indicate that SRC-1 plays an important role in ER-mediated growth of breast cancer cells. Overexpression of SRC-1 potentiates E2-stimulated growth of MCF-7 breast cancer cells in accordance with an increase in the expression of estrogen-responsive genes (Tai et al., 2000). SRC-1 has also been reported to specifically promote breast cancer metastasis, possibly by promoting migration and invasion by enhancing PEA3 mediated transcriptional activation of Twist, a master regulator of metastasis (Qin et al., 2009). Furthermore, SRC-1 may promote metastasis in mammary tumors by facilitating Ets-2 mediated HER-2/neu expression and activating colony stimulating factor-1 expression to recruit macrophages to mammary tumors in mice (Wang et al., 2009).

2.2 SRC-2/TIF-2

The human SRC-2/TIF-2 gene is located on the long arm of chromosome 8 at position 8q21 (Kalkhoven et al., 1998). Knock-out experiments indicate that SRC-2/TIF-2 are of special relevance to fertility and fat metabolism where SRC-2/TIF-2$^{-/-}$ mice have defective spermatogenesis, testicular degeneration, placenta hypoplasia, male and female hypofertility, higher lipolysis in white fat, higher energy expenditure in brown fat and resistance to obesity (Gehin et al., 2002; Picard et al., 2002). Increased levels of SRC-2/TIF-2 mRNA have been observed in intraductal and invasive carcinoma compared to normal breast tissue (Kurebayashi et al., 2000; Haugan Moi et al., 2010). Knockdown of SRC-2/TIF-2 has been shown to inhibit growth of MCF-7 breast cancer cells due to decreased cell proliferation, increased apoptosis and reduced ER mediated transcriptional activity (Cavarretta et al., 2002; Karmakar et al., 2009). However, overall SRC-2/TIF-2 is the least studied of the coactivators in breast cancer.

2.3 SRC-3/AIB1

The third member of the SRC family, SRC-3/AIB1, is located on the long arm of chromosome 20 at position 20q12 in humans and was first identified as a gene amplified and

overexpressed in several human breast cancer cell lines (Anzick et al., 1997). The *SRC-3/AIB1* gene is found to be amplified in 5-10% of breast cancer cases, and SRC-3/AIB1 are overexpressed at mRNA and protein level in 20-60 % of breast cancer patients (Anzick et al., 1997; Takeshita et al., 1997; Bautista et al., 1998; Murphy et al., 2000; Bouras et al., 2001; List et al., 2001; Hudelist et al., 2003; Zhao et al., 2003). Female SRC-3/AIB1$^{-/-}$ mice have significantly lower levels of estrogen and delayed mammary gland development, indicating a proliferative role of this coactivator in breast tissue (Xu et al., 2000). In transgenic mice, overexpression of SRC-3/AIB1 leads to development of tumors in several organs including breast, in addition to increased expression of the insulin-like growth factor-1 (IGF-1) and activation of intracellular pathways suggesting that SRC-3/AIB1 is acting as an oncogene (Torres-Arzayus et al., 2004). The oncogenic potential of SRC-3/AIB1 has been ascribed to mechanisms such as enhanced interaction between ER and the *cyclin D1* promoter, hence leading to increased levels of cyclin D1 and stimulation of cell cycle progression (Planas-Silva et al., 2001). Conversely, cyclin D1 expression has been shown to be reduced in SRC-3/AIB1 knock-out cells (Karmakar et al., 2009), and mice with reduced SRC-3/AIB1 expression have a decrease in epithelial proliferation associated with a reduction in cyclin expression (Fereshteh et al., 2008). Overexpression of SRC-3/AIB1 also stimulates the Akt signaling pathway which promotes cell growth (Torres-Arzayus et al., 2004; Zhou et al., 2003). Matrix metalloproteinases (MMPs) are zink-dependent enzymes involved in the degradation of extracellular matrix and are essential to the metastatic process. Experimental evidence suggests that SRC-3/AIB1 promotes breast cancer metastasis by stimulating the transcription factor PEA3 to enhance expression of MMP2 and MMP9 (Qin et al., 2008).

2.4 Regulation of SRCs expression during endocrine treatment

Most studies seem to indicate higher levels of the SRCs in malignant breast tumors compared to normal breast tissue. However, the expression levels of the SRCs have been shown to change during endocrine treatment in breast cancer. In a clinical study of preoperative tamoxifen treatment for 4 weeks using tamoxifen doses from 1 to 20 mg/daily, we found the mRNA levels of all three SRCs to be significantly upregulated in tamoxifen treated normal and malignant breast tissue compared to samples from untreated patients. The increase in coactivator mRNA expression was especially evident for SRC-3/AIB1 (Haugan Moi et al., 2010). In a clinical study on neoadjuvant treatment with aromatase inhibitors in locally advanced breast cancer, we also found the mRNA levels of coactivators in tumors to increase during treatment, especially for SRC-1 (Flågeng et al., 2009). This is in line with *in vitro* studies. E2 has been shown to repress SRC-3/AIB1 mRNA and protein expression in MCF-7 human breast cancer cells primarily by suppressing SRC-3/AIB1 gene transcription (Lauritsen et al., 2002). Conversely, total SRC-3/AIB1 mRNA levels were increased when MCF-7 breast cancer cells were treated with the antiestrogen 4-hydroxytamoxifen. 4-hydroxtamoxifen has also been shown to increase the stability and hence steady-state levels of SRC-1 and SRC-3/AIB1 proteins in a MCF-7 breast cancer-derived cell line (Lonard et al., 2004). We also found an increase in the mRNA levels of HER-2/neu and a positive correlation between SRC-1 and HER-2/neu in human breast tissue treated with aromatase inhibitors. This finding is interesting in light of *in vitro* assays suggesting that ER and HER-2/neu compete for the coactivator SRC-1. Under antiestrogenic conditions, SRC-1 will be released from the ER and may instead bind to the HER-2/neu enhancer and facilitate transcription of HER-2/neu, leading to increased expression of HER-2/neu under estrogen deprived conditions (Newman et al., 2000).

3. SRCs and endocrine treatment in breast cancer: Molecular mechanisms

The regulation of SRCs during endocrine treatment is especially interesting since the coactivators are directly involved in the molecular mechanisms underlying the antiestrogenic effects. The natural ligand of ER, estrogen, is converted from androgens by the enzyme aromatase. Endocrine treatment of ER positive breast cancer includes aromatase inhibitors or the SERM tamoxifen. Aromatase inhibitors block the synthesis of estrogens by binding to and suppressing the aromatase enzyme that converts androgens to estrogens. Tamoxifen binds to the ER and functions as an antagonist in breast tissue and prevents estrogen from binding to the ER. The net effect of both therapeutic regiments is to block ER-dependent transcriptional regulation of genes and prevent proliferation (Fig. 2).

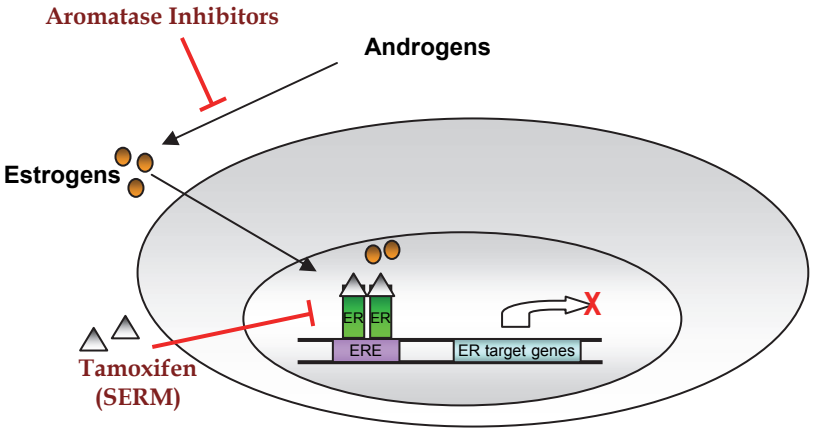

Fig. 2. Schematic presentation of the mechanisms of action of endocrine treatment in breast cancer using tamoxifen or aromatase inhibitors.

3.1 The SERM tamoxifen

Tamoxifen is a synthetic estrogen antagonist which has been in clinical use for over 30 years. While the success of tamoxifen in breast cancer therapy is based on its ER antagonistic effects in malignant breast tissue, tamoxifen demonstrates ER agonistic effects in other organ systems such as bone and liver. ER appears to bind to corepressors in the presence of SERMs in breast tissue, while coactivator recruitment is favored when E2 is bound to ER. Upon binding to ER, SERMs inhibit ER transcriptional activity by competing with E2 for the binding site and by blocking the AF-2 activity of ER (Shiau et al., 1998; Brzozowski et al., 1997). The potent ER antagonistic metabolite 4-hydroxytamoxifen induces a displacement and rotation of the receptor's helix 12. The helix 12 then binds to the hydrophobic pocket via a sequence resembling the NR box of the coactivators, and thereby inhibits coactivator recruitment (Brzozowski et al., 1997; Shiau et al., 1998). The binding of 4-hydroxytamoxifen instead favors recruitment of the two corepressors silencing mediator for retinoid and thyroid hormone receptor (SMRT) and nuclear receptor corepressor (NCoR). These corepressors are associated with histone deacetylase activity and inhibit ER regulated gene transcription (Webb et al., 2003; Fleming et al., 2004a).

However, tamoxifen may exert ER agonistic effects depending on the coactivator context. For example, it has been shown that overexpression of SRC-3/AIB1 and the growth factor HER-2/neu increases the ER agonistic properties of tamoxifen (Shou et al., 2004) and that tamoxifen resistance develops when SRC-3/AIB1 is high and the transcriptional repressor paired box 2 (PAX2) is low in breast cancer cells (Hurtado et al., 2008). Elevated expression of SRC-1 in the uterine derived Ishikawa cell line increases the agonist behavior of 4-hydroxytamoxifen, whereas lower expression of SRC-1 in MCF-7 cells contributed to an ER-antagonistic behavior of tamoxifen (Shang & Brown., 2002). Studies have shown that the estrogenic effects of tamoxifen can be mediated by the constitutive active AF-1 domain of ER which can be stimulated by several mechanisms, including high levels of coactivators (Webb et al., 1998). Hence, the levels of SRCs may determine the response to tamoxifen treatment, at least *in vitro*.

3.2 Aromatase inhibitors

Aromatase inhibitors work by blocking the estrogen synthesis and depriving the breast cancer cells of this important growth factor. In premenopausal women, estrogens are primarily synthesized by the granulose cells in the ovaries, but aromatase activity and conversion of androgens to estrogens also take place in tissues such as subcutaneous fat, breast tissue and bone which are the primary sources of estrogens after menopause. Aromatase is a cytochrome P450 enzyme where the haem protein binds the androgen and catalyzes the formation of the phenolic A-ring which is characteristic for estrogens. Type 1 aromatase inhibitors such as formestane and exemestane, also known as steroidal inhibitors, are analogues to androstenedione and work by competitive binding to the active site of aromatase. Type 2 aromatase inhibitors include the first generation compound aminoglutethimide, the second generation drug fadrozole and the third generation compounds anastrozole and letrozole, which are widely used clinically. These non-steroidal aromatase inhibitors work by binding to an iron atom in the haem group of aromatase and have proved very effective in inhibiting aromatase activity. In the absence of agonist, the ER will be locacted in the cytoplasm associated with heat shock protein (hsp), and dimerization, conformational changes and coactivator recruitment will be inhibited, hence leading to reduced transcription of ER-regulated genes. However, resistance to aromatase inhibitors does occur. In the frequently used cellular model system for resistance to aromatase inhibitors, breast cancer cells are grown in estrogen-deprived conditions for 1-6 months. These long-term estrogen deprived cells (LTED) develop enhanced sensitivity to E2 (Masamura et al., 1995; Santen et al., 2005). This hypersensitivity is associated with upregulation of ERα and the mitogen activated protein kinases (MAPKs) (Jeng et al., 1998; Jeng et al., 2000). The MAPKs are found downstream of several growth factor receptors including HER-2/neu and could phosphorylate and influence the activity of the SRCs, but also the ER. Accumulated evidence points to an important crosstalk between ER and growth factor pathways where posttranslational modifications of the SRCs are involved. These modifications could influence not only SRC activity, but also the effect of endocrine treatment in breast cancer over time.

4. SRCs and growth factor signaling

4.1 Posttranslational modifications of SRCs with functional aspects

The SRCs are components and targets of multiple cell signaling pathways that modulate their activity. Extracellular stimuli such as hormones, growth factors and cytokines induce a

variety of posttranslational modifications of SRCs, including acetylation, methylation, phosphorylation, ubiquitination and sumoylation. These modifications influence the SRCs transcriptional activity and/or the SRC protein levels and stability (Baek & Rosenfeld, 2004; Li & Shang, 2007; Xu et al., 2009).

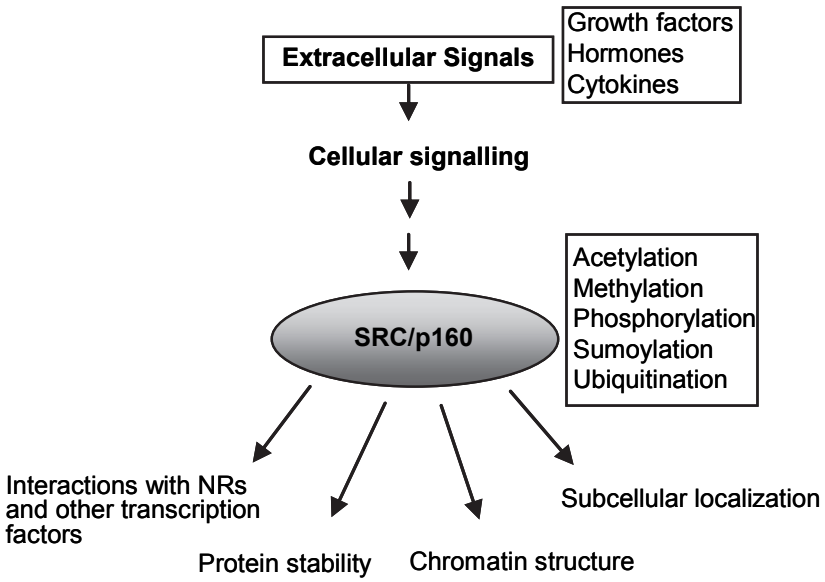

Fig. 3. Functional aspects of posttranslational modifications of the SRCs.

Phosphorylation of coactivators modulates ER-dependent gene transcription by regulating coactivator function in various ways. Three SRC-1 phosphorylation sites with corresponding kinases have been identified (S395, T1179 and S1185), one SRC-2/TIF-2 (S736) and sixteen SRC-3/AIB1 phosphorylation sites (T24, S505, S543, S601, S857, S860, S867, S1033, S1042, S1048, T1059, S1062, T1064, T1067, T1114 and Y1357) (Bulynko & O'Malley, 2011). Comparison of these sites reveals little conservation of sequences among the SRCs, indicating that phosphorylation is a significant determinant of the specificity of the SRCs (Wu et al., 2005).

Phosphorylation may influence the function and acitivity of the SRCs. It is shown *in vitro* using COS-1 cells that positions S395 and T1179/S1185 of SRC-1 are phosphorylated by the MAPK family members ERK1 and ERK2 (Rowan et al., 2000b) where MAPK-mediated phosphorylation on T1179 and S1185 has been shown to increase the affinity of SRC-1 for androgen receptor (AR) in prostate cancer cells (Ueda et al., 2002; Gregory et al., 2004). ERK2 may also phosphorylate SRC-3/AIB1 *in vitro* which stimulates the recruitment of p300 and associated histone acetyltransferase activity (Font de Mora & Brown, 2000). cAMP regulated phosphorylation of SRC-1 occurs through an indirect pathway in which protein kinase A (PKA) induces the activity of ERK1 and ERK2 (Rowan et al., 2000a). SRC-3/AIB1 phosphorylation-defective mutants exhibit reduced ability to interact with ER compared to wild type SRC-3/AIB1, both in the absence and presence of E2 (Wu et al., 2004). Epidermal growth factor (EGF)-induced activation of ER-, progesterone receptor (PR)- and AR-

dependent transcription is shown to be regulated through phosphorylation of SRC-2/TIF-2 at S736 by the EGF-activated ERK MAPK and p38MAPK which stimulate SRC-2/TIF-2 coactivator function (Lopez et al., 2001; Gregory et al., 2004; Frigo et al., 2006).

Phosphorylation and dephosphorylation of proteins also regulate the nuclear import and export by modifying the nuclear localization signals (NLS) and nuclear export signals (NES) of the proteins (Whitmarsh & Davis, 2000). The sequence of the bHLH domain of the SRCs has been shown to be important for their nuclear localization. SRC-1 and SRC-3/AIB1 contain a conserved bipartite NLS in their bHLH-PAS domain (Amazit et al., 2003; Li et al., 2007). Furthermore, specific residues in the NLS of SRC-3/AIB1 are identified to signal proteasome-dependent turnover of SRC-3/AIB1 in the nucleus (Li et al., 2007). SRC-1 also contains a non-conserved sequence localized in its C-terminal region that is suggested to serve as a NES. The return of SRC-1 to the cytoplasm is proposed to be involved in termination of hormone action (Amazit et al., 2003).

Phosphorylation does not only influence the activation and subcellular localization of the SRCs, but also regulate the ubiquitination and degradation of the coactivators. Phosphorylated SRCs are suggested to be targets for enzymes in the ubiquitin-proteasome pathway. The ubiquitin-proteasome degradation pathway is regarded as an important mechanism to control the steady state levels of SRCs, thereby modulating growth responses to various growth-promoting factors (Lonard & O'Malley, 2005). Retinoic acid-induced phosphorylation of SRC-3/AIB1 by p38MAPK at S860, and phosphorylation at S505 by Akt/protein kinase B (PKB)-activated glycogen synthase kinase-3 (GSK3) have been shown to mediate SRC-3/AIB1 degradation (Gianni et al., 2006; Wu et al., 2007). On the other hand, atypical PKC-induced phosphorylation of the C-terminal region of SRC-3/AIB1 was reported to increase its stabilization by protecting it from proteasome-mediated degradation leading to an increased estrogen-induced breast cancer cell growth (Yi et al., 2008).

Growth factor pathways regulate SRC function not only through phosphorylation. We found activation of the cAMP/PKA pathway to stimulate association of SRC-2/TIF-2 with an ER-transcription complex prior to its degradation by the ubiquitin-proteasome system (Fenne et al., 2008). MCF-7 breast cancer cells were transfected with an expression plasmid encoding HA-GRIP1, the rodent homologue to SRC-2/TIF-2, along with the luciferase reporter construct ERE-TATA-luc. Cells were treated with cAMP analog and cAMP-elevating agents for different time-lengths and after 48 hours the cells where lysed and subjected to luciferase assay. A time-dependent regulation of cAMP/PKA on SRC-2/TIF-2 coactivator function was observed (Fig. 4A). PKA is activated when hormones bind to a G-protein coupled receptor (GPCR). The activated receptor interacts with adenylyl cyclase (AC) which catalyses the conversion of ATP to cAMP, further activating the cAMP dependent PKA (Fig. 4b). PKA can regulate SRC-2/TIF-2 coactivator function in a time-dependent matter. Short-term treatment stimulated SRC-2/TIF-2 coactivator function, whereas long-term treatments inhibited SRC-2/TIF-2 function due to ubiquitin-proteasome-mediated degradation (Hoang et al., 2004; Fenne et al., 2008).

All three SRCs can also be modified by site-specific sumoylation of lysine residues in their respective NIDs (Kotaja et al., 2002; Chauchereau et al., 2003). Sumoylation of SRC-2/TIF-2 has been shown to increase its coactivation of AR by enhancing their interaction (Kotaja et al., 2002). Conversely, sumoylation of SRC-1 increases its interaction with the PR and leads to prolonged retention of SRC-1 in the nucleus (Chauchereau et al., 2003). In contrast to SRC-1 and SRC-2/TIF-2, sumoylation of SRC-3/AIB1 seems to attenuate its coactivation function (Wu et al., 2006).

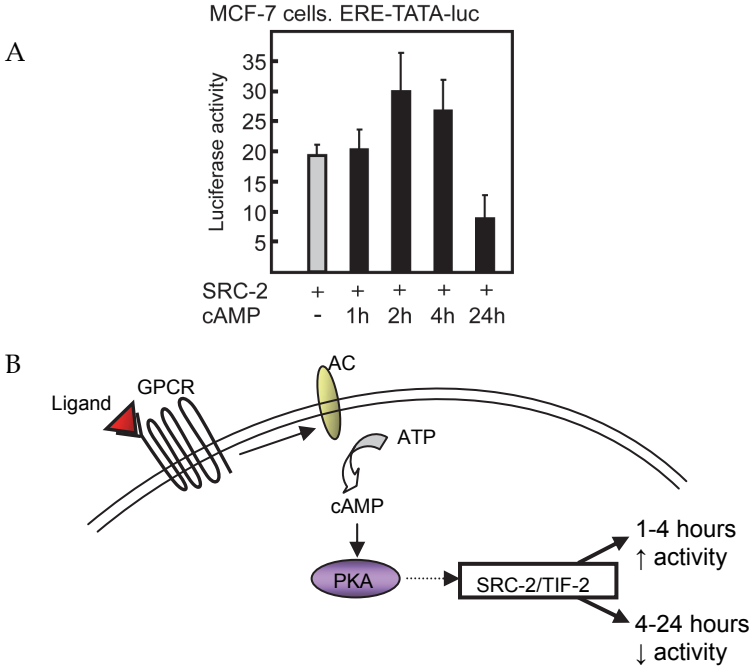

Fig. 4. cAMP-PKA signaling influence SRC-2/TIF-2 function in a time-dependent manner.

4.2 SRCs, growth factor signaling and response to endocrine therapy

The SRCs are regulated by post-translational modifications by kinases found downstream in growth factor signaling pathways often activated in cancers, such as the MAPKs operating downstream of HER-2/neu. Posttranslational modification can stabilize and functionally activate the SRC proteins, a mechanism which has been shown to contribute not only to ER-agonstic effects of tamoxifen, but also to estrogen hypersensitivity and resistance to aromatase inhibitors. *In vitro* it has been shown that tamoxifen resistance with loss of ER antagonistic effects develops when SRC-3/AIB1 is high and the transcriptional repressor PAX2 is low in breast cancer cells (Hurtado et al., 2008). Conversely, dissociation of SRC-3/AIB1 from ER restores tamoxifen's antagonistic effect in resistant breast cancer cells and inhibits further breast cancer cell growth (Planas-Silva et al., 2001; List et al., 2001).

Clinically, studies have shown an association between SRC-1 and reduced disease-free survival in breast cancer patients with locally advanced disease treated with endocrine therapy (Al-azawi et al., 2008; Redmond et al., 2009). During neoadjuvant treatment with aromatase inhibitors, we found higher levels of SRC-1 mRNA levels during treatment, especially in tumors that responded to treatment (Flågeng et al., 2009). Low expression of SRC-1 combined with high ERβ expression has been found to be a good prognostic indicator to endocrine treatment in breast cancers (Myers et al., 2004). However, the clearest association between high levels of SRCs and poor clinical outcome has been found in tumors also overexpressing HER-2/neu. Patients with tumors overexpressing HER-2/neu in

combination with SRC-3/AIB1 or SRC-1 undergoing tamoxifen treatment show reduced sensitivity to endocrine therapy, greater risk of disease recurrence and reduced disease-free survival (Fleming et al., 2004b; Osborne et al., 2003). Overexpression of SRC-3/AIB1 and HER-2/neu in breast tumors is associated with disease recurrences and poor prognosis. This could be linked to the HER-2/neu-mediated activation of MAPK and Akt which causes phosphorylation of SRC-3/AIB1 and ER, resulting in transcriptional activation and cell proliferation. Activation of Akt has also been shown to stabilize SRC-3/AIB1 by inhibiting GSK3 (Wu et al., 2007) whereas PKA-induced resistance to tamoxifen is associated with an altered orientation between ER and SRC-1 (Zwart et al., 2007). Overall, the SRCs can be targeted by central growth factor pathways mediating pro-survival signals and stimulating proliferation. The SRCs close functional relationship with the ER makes posttranslational modifications of the SRCs important points of crosstalk between ER and growth factor signaling pathways during endocrine treatment in breast cancer (Fig. 5).

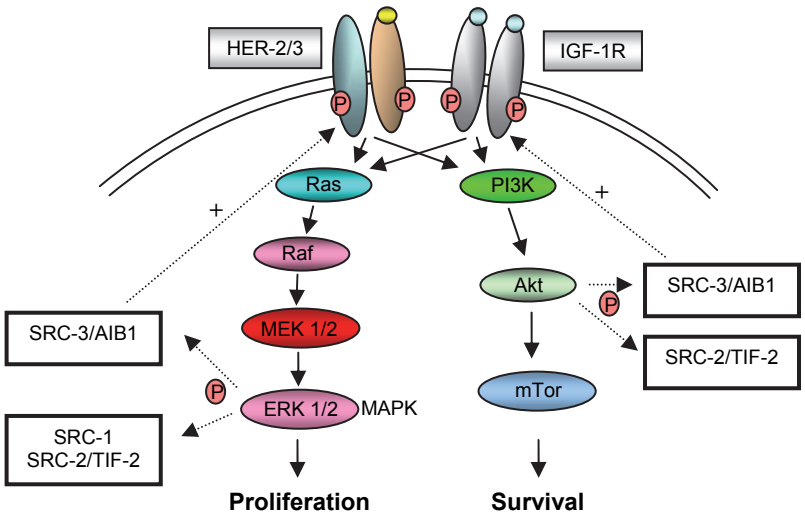

Fig. 5. Cross-talk between growth-factor signaling pathways and SRCs in breast cancer. Ligand-activated growth factor receptor dimers including the human epidermal growth factor receptor-2 and -3 (HER-2/3) and the insulin-like growth factor-1 receptor (IGF-1R) are phosphorylated at intracellular domains and signal both through the MAPK and the phosphatidyl-inositol 3-kinase (PI3K) signaling pathway. ERK 1 and 2 may phosphorylate SRC-1, SRC-2/TIF-2 and SRC-3/AIB1. Akt may phosporylate SRC-2/TIF-2 and SRC-3/AIB1. SRC-3/AIB1 is a modulator increasing the activity and signaling both through HER-2 and IGF-1R leading to cell growth.

5. Conclusion

Most human breast cancers express ER which belongs to the family of nuclear receptors and is a ligand-regulated transcription factor. Endocrine treatment involves blocking the ER with a selective ER modulator such as tamoxifen or inhibiting estrogen synthesis using

aromatase inhibitors. The SRCs are crucial to ER mediated effects and their expression level and activity have been shown to dictate the effect of ER on gene expression to a large extent. SRC-1, SRC-2/TIF-2 and SRC-3/AIB1 are expressed in normal and malignant breast tissue where SRC-3/AIB1 is now considered to be an oncogene. SRC-1 and SRC-3/AIB1 may promote metastasis in mammary tumors by enhancing the transcriptional activation of regulators of metastasis such as Twist and MMPs. The expression levels of the SRCs are influenced by endocrine treatment, an observation which may be of relevance to the treatment response to endocrine therapy over time. We found the mRNA levels of the SRCs, especially SRC-3/AIB1, to be significantly upregulated in both normal and malignant breast tissue after 4 weeks of tamoxifen in the 1-20 mg dose range. The mRNA expression of SRC-1 has also been shown to increase significantly in a clinical study of neoadjuvant treatment with aromatase inhibitors for 14-16 weeks, especially in the subgroup of patients achieving an objective treatment response. This is in line with *in vitro* studies in MCF-7 cells showing that estrogens suppress the mRNA levels of SRC-3/AIB1 by suppressing SRC-3/AIB1 gene transcription whereas 4-hydroxytamoxifen increases the SRC-3/AIB1 mRNA expression level. The importance of the expression level and functional activation of the SRCs during endocrine treatment is evident from cellular assays on tamoxifen treatment. High levels of coactivators relative to corepressors may lead to ER agonistic effects by 4-hydroxytamoxifen. Further, posttranslational modification of both coactivators and ER can lead to altered molecular conformations, intracellular relocation, stabilization and ubiquitination which would influence the activity and stability of the SRCs, as shown for the PKA-mediated regulation of SRC-2/TIF-2. In several clinical trials the levels of coactivators have been found of relevance, not only to the response to endocrine treatment, but also to long term clinical outcome. High protein levels of SRC-1 have been shown to be associated with reduced disease-free survival, both in untreated and tamoxifen treated patients, whereas elevated mRNA expression levels of SRC-3/AIB1 have been associated with high tumor grade and shorter disease-free and overall survival. Tumors undergoing tamoxifen therapy and overexpressing HER-2/neu in combination with SRC-3/AIB1 are more likely to be tamoxifen resistant and are associated with reduced disease-free survival. High expression of HER-2/neu in combination with SRC-1 has also been associated with a greater risk of recurrence on endocrine treatment. In summary, SRCs are expressed in normal and malignant breast tissue and they have a crucial role in mediating the effect of endocrine treatment in breast cancer. The expression levels of SRCs are regulated by endocrine treatment and their functional role is modified by posttranslational modifications mediated by growth factor pathways involved in breast cancer development and endocrine resistance. Further research on SRCs and their role in the crosstalk between ER and growth factor pathways during endocrine treatment is important to improve breast cancer therapy.

6. References

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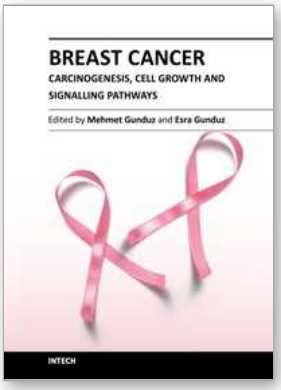

Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways

Edited by Prof. Mehmet Gunduz

ISBN 978-953-307-714-7
Hard cover, 732 pages
Publisher InTech
Published online 30, November, 2011
Published in print edition November, 2011

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Line L. Haugan Moi, Marianne Hauglid Flågeng, Ingvild S. Fenne, Jennifer Gjerde, Ernst A. Lien and Gunnar Mellgren (2011). Steroid Receptor Coactivators and Their Expression, Regulation and Functional Role in Endocrine Responsive and Resistant Breast Cancer, Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways, Prof. Mehmet Gunduz (Ed.), ISBN: 978-953-307-714-7, InTech, Available from: http://www.intechopen.com/books/breast-cancer-carcinogenesis-cell-growth-and-signalling-pathways/steroid-receptor-coactivators-and-their-expression-regulation-and-functional-role-in-endocrine-respo

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