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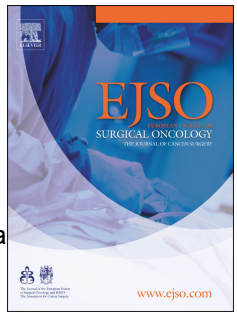

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The role of steroid receptor coactivator - 3 (SRC-3) in human malignant disease

Ondrej Gojis[1,2,3], Bharath Rudraraju[3], Constatine Alifrangis[3], Jonathan Krell[3], Pavla Libalova[1], Carlo Palmieri[3]

[1]Department of Gynaecology and Obstetrics, Third Faculty of Medicine, Charles University, Ruska 87, Prague 10, 100 00, Czech Rep., [2]Department of Pathology, Third Faculty of Medicine, Charles University, Ruska 87, Prague 10, 100 00, Czech Rep., [3]Cancer Research UK Laboratories, Department of Oncology, Imperial College London-Hammersmith Campus, Du Cane Road, London, W12 0NN, UK

To whom correspondence should be addressed:

Dr Carlo Palmieri

Cancer Research UK Laboratories

Department of Oncology,

Imperial College London,

Du Cane Road,

London, W12 0NN, UK

Tel No:+44 20 8383 5828

Fax No:+44 20 8383 5830

Email: c.palmieri@imperial.ac.uk

Abstract

Background

The p160 steroid receptor coactivator (SRC) family are critical to the transcriptional activation function of nuclear hormone receptors. A key member of this family is SRC-3, initially found to be amplified and expressed in breast cancer it has subsequent been shown to be expressed in malignant disease arising from a wide range of other organs. An understanding of the potential role of SRC-3 in the pathogenesis and its possible prognostic role in a broad range of tumours will improvement our general understanding of carcinogenesis as well as potentially leading to a new prognostic marker as well as new therapeutic targets.

Methods

Relevant papers were identified by searching the PubMed and MEDLINE databases for article published until 28th February 2009. Only articles published in English were considered. The search terms included "SRC-3", ''AIB1'' in association with the following terms: "human", "cancer" and "malignant disease". The search focused on malignant disease arising outside of the mammary gland. Full articles were obtained and references were checked for additional material when appropriate.

Results

SRC-3 is amplified and expressed in a wide spectrum of human maligant diseases and appears to be a potential prognostic marker in a number of different tumours.

Conclusion

SRC-3 appears to be implicated in the possible risk of developing prostate and ovarian cancer. Its presence appears to be a marker of aggressive disease. Further research is required to determine its predicitive and prognostic utility given the relative paucity of studies for each specific malignant disease.

Key words: Human, Malignant disease, SRC-3, AIB1

Nuclear Receptors

Nuclear receptors are transcription factors that bind DNA at specific sites to control gene transcription. Nuclear receptors upon ligand binding undergo conformational changes enabling dimersisation and subsequent binding to specific regulatory DNA sequences/response elements upstream of the target genes. The activated receptor, through interactions with coactivator proteins, directs the assembly and stabilization of a preinitiation complex that ultimately conducts the transcription of the target genes.[1] The SRC/p160 family is a key coactivatior family and consist of three members steroid receptor coactivator-1 to 3 (SRC-1;SRC-2/TIF-2/GRIP-1 & SRC-3), and there key role is to act as a bridges between nuclear receptors, other coactivators and the basal transcription machinery.

Steroid receptor coactivator protein-3

Steroid receptor coactivator protein-3 (SRC-3) was identified from an amplified region on the long arm of chromosome 20 (20q) in breast cancer tissue and later named AIB1, it was subsequently shown to be a member of the SRC family. SRC-3 mediated the not only the transctriptional activity of nuclear receptors but also other transcription factors such as E2F1.[1,2]

SRC-3 is a phosphoprotein which is phsophorylated by a number of tyrosine kinases as well as estradiol.[3] Phosphorylation results in SRC-3 becoming a potent transcriptional activator, as well as differential gene expression and modification of its oncogenic potential.There are two phospho-sites that are targeted by phosphatases which prevents protesome dependent turnover of SRC-3.

Structure of Steroid receptor coactivator protein-3

Structurally SRC-3 consists of an N-terminal region containing a basic helix-loop-helix (bHLH) region and a PAS [period (Per), Aryl hydrocarbon receptor (AhR), and single-minded (Sim)] motif, which serves as a protein interaction surface for other types of DNA binding transcription factors. A 'C region' between the bHLH and PAS domain has been linked to the turnover and degradation of SRC-3. The receptor-interaction domain (RID) mediates direct interactions with nuclear receptors in a ligand dependent manner, with the conserved motif of three LXXLL (L, leucine; X, any amino acid) being responsible for this interaction with ligand-bound nuclear receptor.The C-terminal domain has histone acetyltransferase (HAT) activity, as well as two intrinsic transcriptional activation domains (AD1 and AD2), as well as a polyglutamate sequence (polyQ). The AD1 region is responsible for interaction with the general transcriptional cointegrators, CBP and p300 while AD2 is responsible for interaction with histone methyltransferases. Figure 1 summarises the structure of SRC-3 and the known phosphosites.

Animal Models

In animal models overexpression of SRC-3 has been shown to result in an increase in tumor incidence in multiple organs including the breast, uterus, lung and pituitary amongst others.[4] While SRC-3 null mice show growth retardation, delay in puberty, reduced reproductive function, mammary gland growth reduction and inhibition of mammary tumourgenesis and prostate cancer development.[5] Therefore SRC-3 is an oncogene and is clearly important in mediating normal growth and development.

Role of SRC-3 in Hormone Dependent Human Cancers

Prostate cancer

Androgen receptor (AR) plays a pivotal role in the pathogenesis of prostate cancer, and given that the activity of AR is modulated by a variety of coactivators the possible role of SRC-3 in prostate cancer has been investigated. [6,7]

Two studies found non-neoplastic tissue to have less SRC-3 expression compared to malignant tissue.[6,7] In both studies none or only weak SRC-3 stromal staining was seen in both the normal and malignant tissue with varying degress of nuclear and cytoplasmic staining reported within malignant tissue.[6,7] SRC-3 positivity was associate with more advanced stage and grade but not PSA level[6], while tumours with a staining index of ≥4 (13%) had a significantly higher rate of proliferation, as measured by Ki-67, as well as a decreased apoptotic index (TUNEL assay) and significant correlation with p-AKT expression[7]. High SRC-3 was associated with a poorer outcome either in terms of siginifcantly shorter time to PSA recurrence[7] or overall survival[6].

These data show that there are differences in the expression of SRC-3 in normal and malignant prostate tissue and that increased SRC-3 expression in prostate cancer is associated with a more aggressive phenotype and poorer outcome. However, no data was presented on the relationship to the androgen receptor or how SRC-3 staining correlated with response to endocrine therapy or the development of endocrine resistant prostate cancer. Further such correlative clinicopathological studies are required to define potential role of SRC-3 as a potential biomarker in prostate cancer.

Endometrial carcinoma

Given endometrial cancer (EC) is a hormone related malignancy with estrogens implicated in its pathogenesis the role of SRC-3 in ECC has been explored in a number of studies [8-11].Gene amplification has been reported in 17% of EC,[8] with SRC-3 levels being significantly higher in malignant tissue compared to normal tissue[9]. In normal endometrium there was no difference between proliferative and secretory endometrium with regard to SRC-3 but it was significantly correlated with PgR as well as with age[9]. Disperate results have been found in the reported real time PCR studies with regard to the relationship of SRC-3 with type of tumour, grade, depth of myometrial invasion and stage, one study finding no significant difference[9] while another reported significant differences in SRC-3 levels based on these parameters[10]. These differences are potentially related to the small numbers, the differences in methodology and heterogenity in the patient population. Only one study reported on survival, with tumours which had a lower SRC-3 mRNA levels having a statistically superior survival at 2 years compared to those with high levels (96% vs 36%; p=0.002)[10], although the cut off for high and low was not defined.

A IHC study of 88 endometrial carcinomas utilise two SRC-3 antibodies (1) a mouse monoclonal (BD) and (2) a goat polyclonal (SC)[11]. The SC antibody showed cytoplasmic staining and failed to detect a band of the correct molecular weight by western blotting in cells transfected with SRC-3[11], raising questions regarding its utility, the results of IHC with this antibody are therefore not discussed.

Utilising the BD antibody and samples with combinations of different histology there were significantly higher staining score in EC compared with carcinoma-associated complex atypical hyperplasia (CA-CAH) and carcinoma-associated normal endometrium (CA-normal), significantly higher staining score was also seen in type II EC as compared with type I EC[11]. SRC-3 expression correlated with perimenopausal and postmenopausal state as well as higher grade tumours (grade II and III) but there was no correlation with surgical stage[11]. The lack of correlation with surgical stage may reflect the small number of stage III/IV in the study (13% in total). One study investigated the possible relationship to other co-factor found a non-significant trend to a decrease in the ratio of SRC-3:SMRT in malignant tissue (p=0.069), this observation is intresting given the possible interaction between co-activatiors and co-repressors and the fact that changes in their relative level may be important in determing gene transcription. Based on the current data it appears that SRC-3 expression is higher in endometrial cancer compared to normal tissue, and that it appears on balance to be associated with more aggressive disease and worse outcome.

Role of SRC-3 in Hormone Dependent Human Cancers

Gain at 20q has been seen in a variety of tumours including gastrointestinal tumours, hepatocellular carcinoma, urological tumours, nasopharyngeal tumours and uterine cancers. In addition, ERα and/or β expression has been documented in a number of these tumours. Given this the potential role of SRC-3 has been investigated in a wide range of tumour types.

Oesophageal squamous cell carcinoma

SRC-3 is amplified in 4.3-4.9% of oesophageal squamous cell carcinomas (OSCC)[12,13], with 46% overexpressing SRC-3 by immunohistochemistry (IHC)[13]. All the FISH positive cases had overexpression by IHC, the majority of the low level gains were also associated with overexpression of the protein (80% of cases) while 41% of cases with no gene amplification showed overexpression of the protein[13]. Overexpression was associated with larger tumours (54% T3-4 stage vs to 35% T1-T2; p=0.008), as well as with greater Ki-67 staining (p= 0.004)[13]. The oncogenic role of SRC-3 in OSCC does not appear to be dependent on estrogen receptor (ERα) and AR given that all tumours were negative for these nuclear receptors[13]. Given that SRC-3 is know to modulate other transcription factors such as E2F transcription factor 1 (E2F1), Ets-2 and PEA3 [2,14,15], and that these transcription factors have been implicated in OSCC[13], further work is needed to investigate the potential link between these transcription factors and SRC-3 and their possible role in the pathogenesis and progresion of OSCC.

Gastric cancer

High level amplification of SRC-3 has been demonstrated in 7% of gastric cancers, while northern blotting revealed overexpression in all cases investigated, both with and without amplification[16]. SRC-3 expression was increased in malignant tissues relative to normal gastric tissue in 40% of cases investigated by semi-quantitvie RT-PCR ($p < 0.05$)[16]. Low level and high level amplification was associated with significantly greater lymph node involvement, more advanced cancer stage and higher incidence of Borrmann type 3 as well as a shorter survival time when compared to

samples with normal gene expression[16]. To date there has been no investigation of the expression of SRC-3 in gastric carcionoma by western blotting or IHC.

Colorectal carcinoma

10% of colorectal cancers (CRC) possess amplification of SRC-3 with 35% being positive by IHC[17]. No expression of SRC-3 was observed in any of the paired normal mucosa or adenoma specimens, and there was no heterogenity in those specimens with paired lymph node or distant metastatic deposit. Nuclear expression of SRC-3 was significantly higher in disease with nodal or metastatic involvement (23% T3N0M0 vs 48% T3 N1 M0/T3 N2 M1; $p<0.05$), but no other significant clinicopathological correlation was demonstrated[17].

Pancreatic cancer

The amplification and expression of SRC-3 in normal and pathological pancreatic tissue revealed amplification in 37% of the pancreatic adenocarcinomas[18], this is the highest level of amplification for any tumour type. Expression of SRC-3 by in situ hybridization (ISH) and IHC revealed that positivity was significantly higher in high grade PIN and adenocarcinoma compared normal tissue[18]. Similarly, there was a significant increase in the number of samples showing low level positivity when low grade PIN was compared to normal tissue by ISH and IHC. This data appears to implicate SRC-3 in the pathogenesis of pancreatic cancer and that it may be a potential diagnostic marker given its differential expression across normal and malignant tissue.

Hepatocellular carcinoma

25% of hepatocellular carcinoma (HCC) have been reported to have amplification of SRC-3 compared to none in normal tissue[19]. Significant differences in amplification were observed between lesions <3cm and those >3cm (3% vs 20% respectively P<0.05), between single nodular and multiple nodular lesions (16% vs. 30%; $P < 0.05$), between primary compared to recurrent disease (29% vs 60%; p<0.05), as well as in primary compared to metastatic disease (23% vs 41%; p<0.05). Where paired specimens of the primary and recurrent lesion were available 33% had amplification in recurrent specimen alone, while in cases with paired primary and metastatic lesion 32% had amplification in metastatic lesion alone[19]. This study suggests that amplification of SRC-3 is a late genetic event in the development of HCC and is associated with recurrent or metatstatic HCC.

Urothelial cancer

SRC-3 amplification has been described in 7% of bladder tumours[20]. IHC of normal bladder mucosa revealed less than 10% staining, and this was subsequently used as the cut off for defining positivity in tumours samples, of which 32.5% were positive. No correlation was found between the expression of SRC-3 with any clinicopathological features including nuclear receptors. However, the expression of Ki-67 was significantly higher in those tumours positive for SRC-3. The overall survival was lower for those with SRC-3 positive tumours compared to those that were negative (45.6 vs 59 mths; $p < 0.001$)[20] and SRC-3 was an independent prognostic factor by multivariate analysis (relative risk 3.571; 95% CI 1724-7.397, p<0.001). These results suggest that SRC-3 expression is a marker of poor outcome in bladder cancer although the potential underlying mechanism is unclear[20].

Upper Respiratory Tract

Nasopharyngeal Carcinoma

Amplification and overexpression of SRC-3 occurs in 7% and 51% of poorly differentiated nasopharyngeal carcinoma (NPC)[21]. Overexpression was significantly associated with large tumours, lymph node postitivity and a high labelling index for Ki-67[21]. Based on this small series it appears that overexpression of SRC-3 leads to a more aggressive phenotype and consistent with other studies it is associated with metastasis. Given ERα, PgR or AR was negative in all samples the mechanism by which SRC-3 modulates the pathogenesis in NPC is not dependent on expression of these nuclear receptors, and therefore likely to involve another transcription factors, as in OSCC[13] .

Central Nervous System

Meningiomas

Given evidence suggests that mengiomas are hormonally dependent, and that they have been shown to express ERα, ERβ and PgR[22] the possible role SRC-3 in meningiomas has been explored. 76% of meninigomas were positive as compared to none of the normal brain tissue[22]. There was variability of expression by type of menigioma, as well heterogentity with regard to ERα and PgR status in the SRC-3 positive samples with 38% being ERα nd PgR positive, 6% ERα positive and PgR negative, 25%ERα negative and PgR positive and 31% ERα and PgR negative. Further, larger and molecular based studies are required to explore this area further as well as investigating any correlation between the presence of SRC-3 and the response

to tamoxifen. Such studies will potentially define the role of SRC-3 in the pathogenesis of menigiomas and its potential utility in determining resposiveness to endocrine therapy.

Role of SRC-3 Polyglutamine Repeat in Ovarian and Prostate Cancer

SRC-3 contains a polymorphic stretch of glutamine-residues within the carboxy-terminus (poly-Q)[23]. The biologic function of these repeats is unclear, however the analogous region of SRC-1 directly interacts with the androgen receptor (AR) to enhance its signaling, and the length of the CAG repeats inversely correlates with transcriptional activity of AR[24]. The possible role of the poly-Q tract has been investigated in ovarian and prostate cancer.

Prostate Cancer

Given the role of AR in prostate cancer,that SRC-3 is a co-activator of AR and the previously documented effects of the polyQ repeat within the AR[25] the possible risk modifying effect of the polyQ repeat in SRC-3 has been investigated [25,26]. A study of Caucasian men found that compared to the presence of at least one polyQ of 29 or the 28/29 genotype there was no increase in the relative risk of prostate cancer for carrier of other alleles[26]. In addition, no clear association was seen for stage at diagnosis/histological grade and the SRC-3 polyQ tract[26]. In a smaller Chinese study relative to the 29/29 genotype, homozygous for the 28 allele had a significant increased risk of prostate cancer (OR, 2.12; 95%CI, 1.09–4.12), while all other genotypes were associated with a non-significant increase in risk[25]. The possible relationship between SRC-3 and AR polyQ was evaluated in both studies. No relationship was seen in the American study[26], while the Chinese study found relative to men with the SRC-3 polyQ genotype 29/29 and a long polyglutamine repeat in AR

(≥23), those with both the <29/<29 AIB1 genotype and a short polyglutamine repeat in AR (<23) had a 2.8-fold increased risk of prostate cancer (OR, 2.78; 95% CI, 1.24–6.26)[25]. The inconsistencies between these studies are likely to be related to a number of factors including ethnicity, which may explain the differences in the prevalence of the 29 allele (65.5% Chinese study vs 47.8% American Study), which may have influenced the results. Differences in the susceptibility, biology of the disease as well stage of the disease in the studies may also explain the differences. With regard to the relationship to AR polyQ both studies utilised difference cut offs to define short versus long repeats and this as well as the other issues listed may explain the inconsistencies in the relationship between SRC-3 and AR. Further larger ethnically diverse studies with complete clinicopathological data are required to investigate the role of the SRC-3 poly Q repeat with regard to prostate cancer risk, as well as effect on disease biology. To date there has been no exploration of the possible influence of the SRC-3 polyQ on response and outcome to endocrine therapy.

Ovarian Cancer

Androgens and the AR have been implicated in the pathogenesis of ovarian cancer although inconsistencies exist with regard to the epidemiological data. Short polymorphisms of the polyQ sequence within AR have been associated with decreased surgical cytoreducibility and poor overall survival in epithelial ovarian cancer[27]. Given this and what is know regarding the analogous region in SRC-1 the possible role of the SRC-3 polyQ tract was explored in ovarian cancer [27]. A short SRC-3 polyQ genotype (≤28 repeats) was associated with a statistically shorter time to disease recurrence compared to those with a long genotype (≥29 CAG repeats; 15.0 versus 30.0 months; P = 0.01)[27], as well as a lower overall survival (57.0 months)

compared to those with a long genotype (median survival not yet reached; P = 0.02). Multivariate analysis identified the presence of a short SRC-3 polyQ genotype as an independent poor prognostic factor for overall survival (P = 0.05) [27]. No data was presented on the possible relationship or effect of the AR polyQ with regard to SRC-3 polyQ. This data suggests that the length of the poly Q tract within SRC-3 can potentially influence disease outcome, the mechanism for this effect maybe via differential activation of nuclear receptors such ER or AR resulting in enhance signalling. However, this study was small and further work is required to expand and confirm this observation.

Conclusion

SRC-3 is a key co-regulator of the actions of a number of transcription factors and has been shown to be important in hormonally regulated cancers which are dependent on the express of ERα or AR. However, its role in the pathogenesis of human malignant disease appears not to be limited to just this sub-group of tumours given its expression in tumours not considered to be classically hormonally regulated. This observation is not entirely unexpected as ERα negative breast cancer has been shown to express SRC-3 and be associated with a poorer overall survival[28] and given SRC-3 is a known co-factor for wide range of transcription factors [2,14,15].

A number of general observations can be made from the published studies of SRC-3, these include its elevated expression in malignant tissue as compared to normal tissue [7,9,16-18,22], its correlation with markers of aggressive disease such as increased Ki-67 staining[13,21], larger tumours [13,19,21], lymph node involvement [16,17,21], more advanced

stage,[16,17], higher expression in metastatic deposit[19], and where correlation has been made with outcome expression it is a marker of poorer outcome[6,7,20].

These observed associations would fit with the known role of SRC-3 in cell motility, which is known to involve focal adhesion turnover and focal adhesion kinase activation[15], as well as its known upregulation of expression of matrix metalloproteinase[15]. As well as its role in cell migration and invasiveness, SRC-3 maintains IGF-I in the circulation[29], as well as mediating the effects of IGF-I induced proliferation, signaling and cell survival[30]. Given that circulating levels of IGF-I are known to be raised in a number of tumour types and IGF-I has been shown to be involved in the biology of a number of tumours as a mitogen and survival factor, the relationship between SRC-3 and IGF-I requires further detailed investigation. Evidence also exists that SRC-3 plays a role in platelet-derived growth factor/vascular endothelial growth factor signaling, which also warrants investigation in human malignant disease given the importance of these tyrosine kinases in human cancer.

However, much of the data with regard to SRC-3 is limited to single papers involving small cohorts which are relatively underpowered therefore further research is required to define accurately the precise role of SRC-3 at a molecular level and its potential diagnostic and prognostic potential of SRC-3, as well as the possible value as a therapeutic target of SRC-3. Furthermore, given that SRC-3 is a phosphoprotein and its activity can be modulated by tyrosine kinases, research to investigate if any of these phosphorylated forms of SRC-3 are of any importance in human malignant disease, and if targeting of SRC-3 phosphorylation could be a useful therapeutic manoeuvre is required.

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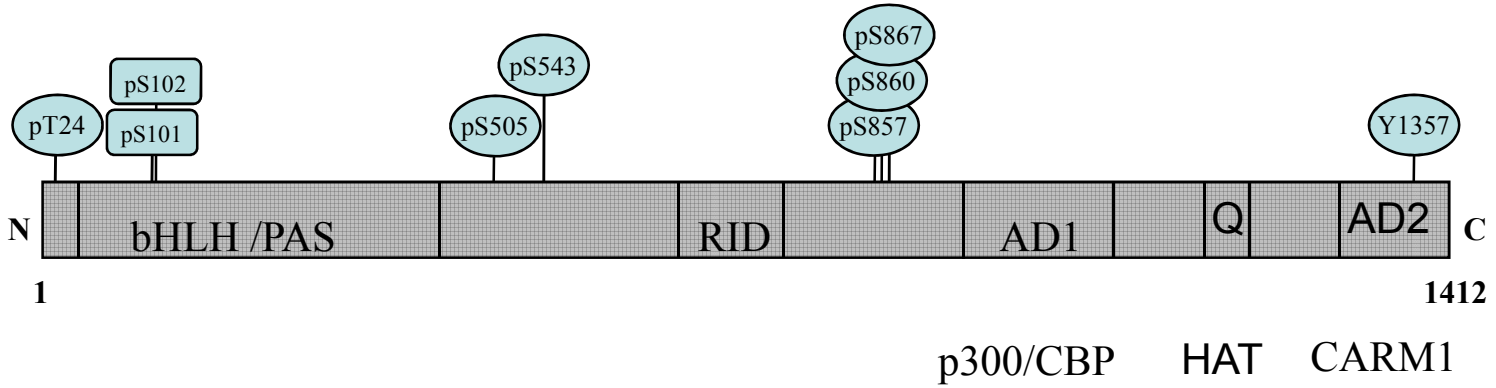

Figure 1 Schematic representation of the structural domains of SRC-3 and known phosphosites

bHLH: basic helix-loop-helix region; PAS:period (Per), Aryl hydrocarbon receptor (AhR), and single-minded (Sim) motif; RID: receptor interacting domain; AD: activation domain1, 2; pT: phosphothronine; pS: phosphoserine;pY:phosphotyrosine; Q: polyglutamate repeat tract. The specific domains for interaction with p300/CBP,coactivator associated arginine Methyltransferase 1 (CARM1), as well as the histone acetyltransferase (HAT) domain, are indicated.