Regulation of gene expression by the RNA-binding protein Sam68 in cancer

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Abstract
Sam68 (Src-associated in mitosis 68 kDa) is the prototypical member of the STAR (signal transducer and activator of RNA) family of RNA-binding proteins. Sam68 is implicated in a number of cellular processes including signal transduction, transcription, RNA metabolism, cell cycle regulation and apoptosis. In the present review, we summarize the functions of Sam68 as a transcriptional and post-transcriptional regulator of gene expression, with particular relevance to cancer.

Introduction
Sam68 (Src-associated in mitosis 68 kDa) belongs to the STAR (signal transducer and activator of RNA) family of RNA-binding proteins that link signalling pathways to RNA processing (for a review, see [1]). STAR family proteins are characterized by at least three functional motifs: a single extended KH [hnRNP (heterogeneous nuclear ribonucleoprotein) K homology] domain embedded within a larger conserved GSG (GRP33/Sam68/GLD1) domain consisting of approx. 80- and 30-amino-acid sequences referred to as the NK (N-terminus of KH) and CK (C-terminus of KH) regions respectively (Figure 1).

The RNA-binding ability of Sam68 is harboured within the GSG domain, which is required for homodimerization and sequence-specific binding to RNA targets. The GSG domain is flanked by a proline-rich WW domain (protein–protein interaction domain containing two conserved tryptophan residues)- and SH (Src homology domain) 3-binding regions, and SH2-interacting tyrosine-rich motifs, which mediate interplay with numerous cell signalling components in response to different stimuli and critically regulate Sam68 function (reviewed in [1]).

Sam68 and RNA processing
A major role for Sam68 is in the regulation of RNA metabolism through RNA processing and export. Sam68 binds non-specifically to poly(U) and poly(A) RNA, and specifically to the high-affinity binding sequences UAAA or UUUA as determined by SELEX (systematic evolution of ligands by exponential enrichment) [2]. The 3′-UTR (3′-untranslated region) contains an AU-rich sequence, and recent work by our group suggests that this region is a target for Sam68 binding (Y. Liu and D.J. Elliott, unpublished work). Although a direct role in translation has not been demonstrated, Sam68 has been implicated in modulating the nuclear export and cytoplasmic utilization of viral mRNAs [3,4].

Despite a predominantly nuclear localization, Sam68 was first identified as the major phosphorylation substrate for Src in the cytoplasm during mitosis. Post-translational modifications can affect Sam68 function, by critically regulating intracellular localization and accessibility to RNA: tyrosine residues are phosphorylated by non-receptor tyrosine kinases (such as Src and Fyn) and subsequently bound by SH2 domains preventing association with RNA [5]; methylation of RG (arginine/glycine)-rich regions by arginine N-methyltransferases is required for RNA export [4]; acetylation of lysine residues within the GSG domain by histone acetyltransferases positively regulates RNA binding [6]; and SUMO (small ubiquitin-related modifier) modification enhances the ability of Sam68 to repress cyclin D1 expression [7].

The above findings suggest that Sam68 behaves as a multifunctional adaptor by linking signalling pathways to the transcriptional and post-transcriptional regulation of gene expression. In particular, Sam68 was shown to induce ERK (extracellular-signal-regulated kinase)-mediated inclusion of CD44 variable exon v5 in response to Ras activation by phorbol ester stimulation [8]. Sam68 was identified as a target for phosphorylation by ERK and was found to bind exonic splice-regulatory elements within CD44 exon v5. Misregulation of CD44 splice variants is implicated in cancer.

More recently, Sam68 was shown to associate with Brm, a component of the SWI/SNF (mating-type switch/sucrose non-fermenting) chromatin remodelling complex, which also favours inclusion of CD44 variant exons [9]. The study...
Figure 1 | Sam68 domain structure
Tripartite GSG domain containing a KH domain, NK region and CK region. Flanking domains contain six consensus proline-rich motifs (P0–P5), RG-rich sequences, and a tyrosine (Y)-rich region close to the C-terminal NLS (nuclear localization sequence).

Figure 2 | A model for Sam68 function in the regulation of gene expression
Distinct functions for Sam68 protein (i) at the promoter with a role in transcriptional co-regulation; and (ii) associated with the C-terminal domain (CTD) of RNAPII, with a role in RNA processing. These functions may be regulated by the post-translational modifications such as phosphorylation (P). MAPK, mitogen-activated protein kinase; RTK, receptor tyrosine kinase; TF, transcription factor.

suggested that Brm and Sam68 may co-operate to slow the rate of RNAPII (RNA polymerase II) on genes regulated by SWI/SNF, thus facilitating recruitment of the splicing machinery to suboptimal splice sites within variable exons [9]. In this way, Sam68 may contribute to cross-talk between cell signalling, transcription and RNA processing.

Sam68 and transcriptional regulation of gene expression
Direct evidence implicating Sam68 in transcriptional regulation of gene expression is limited: binding of Sam68 to hnRNP K inhibits the function of hnRNP K in transcriptional activation of a reporter driven by the CT promoter element of the proto-oncogene c-myc, through direct protein–protein interaction [10]. Sam68 has been noted to repress various mammalian and viral promoter constructs, and potently inhibit the transcriptional activity of the multifunctional adaptor CBP [CREB (cAMP-response-element-binding protein)-binding protein] in a GAL4-dependent reporter system independent of its RNA-binding ability [11]. In these scenarios, it is thought that Sam68 may behave as a competitive inhibitor of positive regulators of transcription.

The role of Sam68 as a transcriptional co-regulator is likely to be promoter- and transcription-factor-dependent: Sam68 has been shown to enhance the transcriptional activity of the AR (androgen receptor), a nuclear hormone receptor transcription factor [12]. This observed effect was seen using a reporter system driven by the steroid hormone-responsive MMTV (murine-mammary-tumour virus) promoter, and was independent of the ability of Sam68 to bind RNA. AR transcription function is important in prostate cancer progression, and Sam68 has been found to be overexpressed in prostate tumours [12,13].

The co-activator function of Sam68 may also extend to other nuclear hormone receptors: Sam68 haploinsufficiency has been shown to delay the onset of mammary tumorigenesis driven by the polyoma middle T-antigen oncogene under transcriptional control of the MMTV promoter [14]. Since ER (oestrogen receptor) signalling is important in breast cancer and the MMTV promoter is also ER-dependent, Sam68 may also be acting as a co-activator of ER-dependent transcription in mammary development and tumorigenesis.

Sam68 and cancer
The evidence implicating Sam68 in cancer is contradictory: Sam68 was initially thought to be a tumour suppressor, but more recently it has been shown to exhibit a pro-oncogenic function (reviewed in [15]). On the one hand, Sam68 overexpression induces G1 growth arrest, and down-regulation of cyclins D1 and E independently of RNA-binding capacity [16]. Sam68 SUMOylation on lysine residues also enhances this ability to repress cyclin D1 expression [7]. However, on the other hand, Sam68 knockdown in prostate cancer cells delays cell cycle progression and reduces proliferation, but causes an accumulation of cyclin D1 [13].

Sam68 is a downstream target of important signalling pathways activated in carcinogenesis such as receptor and non-receptor tyrosine kinases and RET (rearranged during transfection) pathways (reviewed in [15]). Since ER signalling and AR signalling are important in breast and prostate cancers respectively, Sam68 may be functioning as a steroid hormone receptor transcriptional co-activator in these tumours. Sam68 may also have a role in progression of these cancers to a hormone-refractory phenotype.

Conclusions
On the basis of current evidence, it is unclear whether Sam68 is a tumour suppressor or classical oncogene. Sam68 appears to have biochemically distinct roles in the cell depending on post-translational modifications as a result of the activation of different signalling pathways in cancer (Figure 2). Further
studies are required to clearly define the functional roles of Sam68 in cancer with the assistance of tissue-specific genetically engineered models to study autochthonous tumours.

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