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FOR THE RECORD

p53 Family members p63 and p73 are SAM domain-containing proteins

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Abstract: Homologs of the tumor suppressor p53, called p63 and p73, have been identified. The p63 and p73 family members possess a domain structure similar to p53, but contain variable C-terminal extensions. We find that some of the C-terminal extensions contain Sterile Alpha Motif (SAM) domains. SAM domains are protein modules that are involved in protein–protein interactions. Consistent with this role, the C-terminal SAM domains of the p63 and p73 may regulate function by recruiting other protein effectors.

Keywords: Sterile Alpha Motif; tumor suppressor

The recently cloned genes, p63 and p73, are close homologs of p53, the most frequently mutated gene associated with human cancer (Kaghad et al., 1997; Yang et al., 1998). p53 serves as a regulator of the cell's genomic damage response pathway, cell cycle arrest in response to cellular stresses, and apoptosis (Levine, 1997). The domain structure of p63 and p73 closely resembles that of p53, with strong sequence similarity in the transactivation, DNA binding, and oligomerization domains (Kaghad et al., 1997; De Laurenzi et al., 1998; Yang et al., 1998). Whereas the p53 gene encodes a unique polypeptide, multiple splice variants are expressed from the p63 and p73 genes, which have different functions. In particular, the β, γ, and δ splice variants of p63 and p73 can mimic some functions of p53 when overexpressed, including oligomerization, activation of promoters containing p53 binding sites, and the induction of apoptosis. However, the α splice variants of p63 and p73 (TAp63α, TA*p63α, ΔNp63α, and p73α), which possess a C-terminal extension beyond the p53 core, show dramatically reduced p53-like function (Jost et al., 1997; Kaghad et al., 1997; De Laurenzi et al., 1998; Yang et al., 1998). Thus, it has been proposed that sequence elements exist within the p63α and p73α C-terminal extensions that regulate the p53-like functions of these proteins (Yang et al., 1998). We have found that a previously unnoticed Sterile Alpha Motif (SAM) domain is encoded within the C-terminal extensions of the α splice variants of p63 and p73.

SAM domains are found in a wide variety of proteins involved in cell signaling including the Eph family of tyrosine kinase receptors (Hirai et al., 1987; Tessier-Lavigne, 1995), the ETS family of transcription factors (Kyba & Brock, 1998), polyhomeotic proteins (Kyba & Brock, 1998), diacylglycerol kinases (Sakane et al., 1996), liprins (Ponting, 1995), the connector enhancer of KSR (Therrien et al., 1998), serine/threonine kinases, adapter proteins, and others (Schultz et al., 1997). SAM domains are known to associate with other SAM domains, forming both homo-oligomers and hetero-oligomers (Kyba & Brock, 1998; Thanos et al., 1999). Abnormal SAM-mediated oligomerization is the cause of many human leukemias (Jousset et al., 1997). In addition, SAM domains can associate with other proteins, such as AF6 and probably protein tyrosine phosphatases (Serra-Pages et al., 1995; Hock et al., 1998; Stein et al., 1998). In this manner, SAM domains may provide the scaffold for the construction of large protein complexes in the cell.

Results and discussion: The SAM domain is encoded primarily within exon 14 in the p63α gene (Yang et al., 1998) and is within exon 13 in p73α gene (Kaghad et al., 1997; De Laurenzi et al., 1998). Differential splicing of these genes causes the SAM domain encoding exons to be spliced out of the p63 and p73 β, γ, and δ splice variants. The domain structure of the α and β splice variants is shown in Figure 1. The SAM domain of p63α (p63-SAM) is composed of residues 502–567, and the SAM domain of p73α (p73-SAM) is composed of residues 485–541. The p63-SAM is 33% identical and the p73-SAM is 29% identical to a previously identified SAM domain from a squid p53 homolog (residues 453–516; Schultz et al., 1997). In addition to the clear sequence relationship, the p63α and p73α SAM domains are compatible with the known structure of the SAM domain (Slupsky et al., 1998; Stapleton et al., 1999; Thanos et al., 1999). As shown in Figure 2, conserved hydrophobic residues that are buried in the structure of the SAM domain from the EphB2 receptor tyrosine kinase are strongly conserved in the p63 and p73 SAM domains. Moreover,

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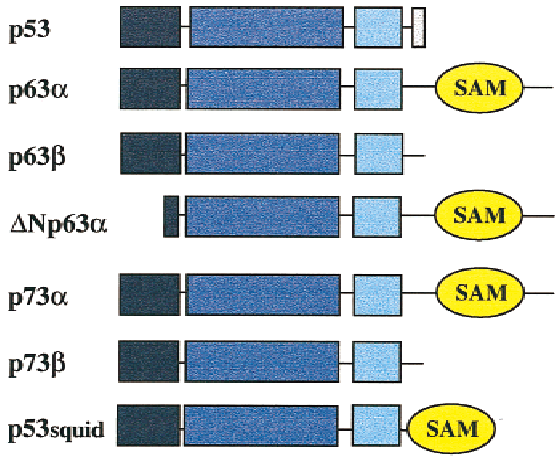

Fig. 1. The domain structure of p53 family members. SAM domains, shown in yellow, are conserved in the α splice variants of p63 and p73 and a squid p53 homologue. The transactivation domain, shown in dark blue, comprises the N-terminal 45 amino acids and interacts with basal transcription machinery in a positive manner. The DNA binding domain, shown in royal blue, consists of approximately 200 amino acids. The oligomerization domain, shown in light blue, forms a tetramer and consists of approximately 30 residues. The gray box at the C-terminus of p53 is a 26 residue basic sequence that aids in DNA binding.

p63-SAM and p73-SAM share conserved residues that are buried in the EphB2-SAM oligomer interfaces.

Although the role of the SAM domain in regulation of p63 and p73 has yet to be elucidated, it is possible that the SAM domain itself mediates negative regulation of p53-like activity. SAM domain-containing splice variants of p63 and p73 do not possess many of the p53-like functions such as oligomerization, activation of promoters containing p53 binding sites, and the induction of apoptosis. As SAM domains are known to bind other proteins, it is possible that the range of interactions could include a region of p63 and p73 that is critical for p53-like function. A similar mechanism is seen in the association of MDM2 with the transactivation domain of p53 (Kussie et al., 1996). In the crystal structures of the EphA4 and EphB2 SAM domains, the SAM domains bind to N-terminal peptide arms (Stapleton et al., 1999; Thanos et al., 1999). It is therefore conceivable that the p63 and p73 SAM domains bind to peptide regions using the same binding pocket. In the absence of direct evidence implicating the SAM domains in negative regulation, however, it is impossible to rule out an independent role for the SAM domains in p63 and p73 function, perhaps involving the recruitment of other factors. Nevertheless, the identification of SAM domains within the α-splice variants provides a new structural and functional context for the design of experiments to elucidate the function of this important family of proteins.

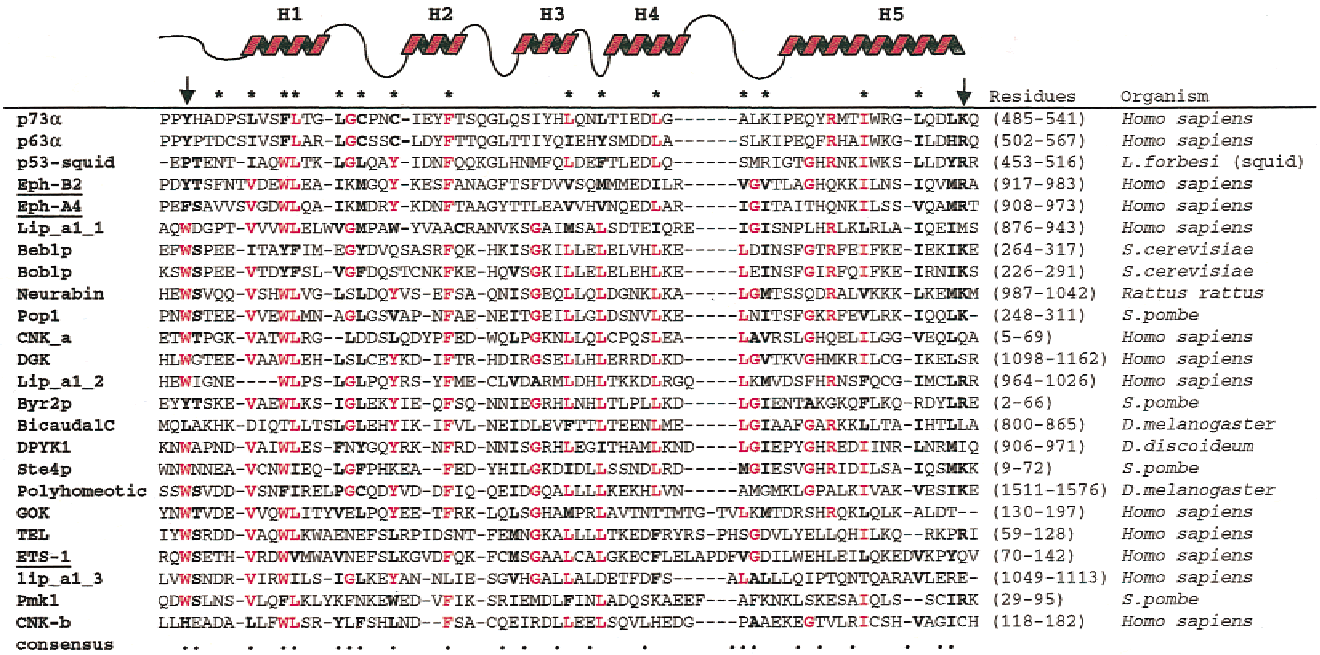

Fig. 2. A multiple sequence alignment of SAM domains. The structure of the EphB2 SAM domain consists of five helices, H1–H5. A multiple sequence alignment was created using the program CLUSTALW. Identical positions in greater than 50% of the sequences are shown in red, and similar positions are shown in bold. Hydrophobic core positions in the Eph-B2 SAM structure (defined as residues with 90% of their surface area buried) are designated with an asterisk. Areas were determined using the program ENVIRONMENTS (Bowie et al., 1991). Key oligomeric interface positions (Thanos et al., 1999) are marked with an arrow. SAM domains whose structures have been solved are underlined. The p63 and p73 SAM domains were found using the program BLASTP 2.0 with BLOSUM-80 substitution matrix with a gap cost of (10,1). The *Loligo forbesi* p53-SAM (residues 453–516) was used as a query sequence. The p73 and p63 sequences both matched the query with an E-value of 1×10^{-8}. The *L. forbesi* p53-SAM was originally identified by Schultz et al. as a SAM domain using the Ste11p SAM (residues 17–83, accession number P23567) as a query sequence (Schultz et al., 1997). The accession numbers of sequences in this alignment are: p73α [Y11416], p63α [AF075430], p53-squid [U43595], EphB2 [D14717], EphA4 [Q03137], Lip a1 1 [U22815], Beblp [P39969], Boblp [P38041], Neurabin [U72994] Pop1 [P38041], CNK [AF100153], DGK [Q16760], Lip a1 2 [U22815], Byr2p [Z98270], BicaudalC [1085137], DPYK [1730077], Ste4p [548999], Polyhomeotic [X63672], GOK [U52426], TEL [Z35761], ETS-1 [X14798], lip a1 3 [U22815], Pmkl [U53872].

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