An Identity Crisis for a Cancer Gene: Subcellular Location Determines ASPP1 Function

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Two recent papers in *Genes and Development* argue that the ASPP1 protein has distinct roles in cell survival, depending upon its subcellular localization, that are determined by a complex interplay with LATS kinase and the YAP transcriptional cofactor.

The ASPP family of proteins was originally identified as playing an obligate role in the transcriptional regulation of the tumor suppressor p53 (see Sullivan and Lu, 2007). ASPP1 and ASPP2 were shown to interact with p53 directly and selectively enhance its transcriptional activity on target genes that play a role in apoptotic outcomes (Samuels-Lev et al., 2001). The iASPP protein antagonizes these effects and contributes to the downregulation of a p53-mediate cell death response (see Sullivan and Lu, 2007). These studies argued in favor of a tumor suppressor role for ASPP1 and ASPP2 in the nucleus. Two recent studies in *Genes and Development* (Aylon et al., 2010; Vigneron et al., 2010) now support the notion that ASPP1 is predominantly cytoplasmic in unstressed cells and that this localization is determined by an interplay with LATS kinases and the YAP transcriptional cofactor.

Both the LATS kinases (LATS1 and LATS2) and YAP are components of the Hippo signaling pathway in mammalian cells (see Hergovich and Hemmings, 2009). Phosphorylation of YAP by the LATS kinases leads to its cytoplasmic localization and targeting for protein degradation via the proteasome (see Bertini et al., 2009). Vigneron et al. now demonstrate that ASPP1 can antagonize the interaction of YAP with the LATS kinases. This prevents YAP phosphorylation on serine 127 and results in its entry into the nucleus, where it contributes to a transcriptional program that enhances survival, notably including the downregulation of the proapoptotic protein Bim (Vigneron et al., 2010). That program involves a role for YAP as a coactivator of the transcription factor TEAD (Zhao et al., 2008). Thus, localization of ASPP1 to the cytoplasm enhances an oncogenic activity of YAP.

In contrast, oncogenic signaling in response to expression of activated Ras stimulates the ability of the LATS kinases to phosphorylate ASPP1 (Aylon et al., 2010). Such modification of ASPP1 results in the translocation of the LATS-ASPP1 complex to the nucleus, where it selectively enhances the transcriptional activity of the tumor suppressor p53 on target genes that are specifically relevant to apoptosis (Aylon et al., 2010). These findings reinforce the idea that nuclear ASPP1 plays a tumor-suppressing role.

Clearly, conventional notions of oncogenes and tumor suppressors are challenged by the findings in these two intriguing studies. The notion that subcellular localization will inform whether a specific protein promotes or inhibits proliferation is exciting. This is especially true when this occurs via distinct mechanisms in each cellular compartment. Further, this has important implications for how a target such as ASPP1 may be used either as a biomarker or a focus for therapeutic intervention. As is often the case, these exciting findings also raise additional, compelling questions about the roles of YAP and ASPP1 in oncogenesis.

While Vigneron et al. focus on the pro-survival effects of localizing YAP to the nucleus, they also note that YAP has previously been shown to serve as a cofactor for the p53 family member p73 (Figure 1) (Vigneron et al., 2010). Occupying specific genes in conjunction with p73, YAP has been shown to contribute to transcriptional regulation that leads to an apoptotic outcome (Strano et al., 2005). Thus, the consequences for the cell upon nuclear localization of YAP will be determined to some extent by the relative importance of its interplay with TEAD (leading to survival) (Zhao et al., 2008) and p73 (promoting cell death) (Strano et al., 2005). Studies in human tumor samples have indicated that YAP is often overexpressed in a subset of cancers (see Bertini et al., 2009). This is more consistent with an oncogenic role for YAP, yet its interactions with p73 need to be more fully understood. p73 is know to act in response to specific agents that trigger genotoxic DNA damage, whereas the studies of Vigneron et al. did not directly address the role of survival signaling of YAP in this context.

Aylon et al. demonstrate a role for the nuclear ASPP1/LATS complex in enhancing the transcriptional activity of p53 specifically on its target genes relevant for apoptosis. Of interest, they note that the outcome of this effect is selective killing of polyploid cells (Aylon et al., 2010). A previous study from the same laboratory demonstrated that abrogation of LATS2 expression promoted the presence of tetraploid cells (Aylon et al., 2006). In their current study, they begin to provide a mechanistic basis for this earlier observation. Nevertheless, it is unclear why polyploid cells would be selectively targeted under these conditions, given that the genes that are being affected should be stimulating cell death regardless of the actual DNA content. It remains to be seen whether there is a novel set of target genes that mediate this effect or else that LATS2 may exert other effects independent of the ASPP1-p53 gene expression program.

In response to oncogenic signaling or mitotic stress, LATS2 translocates from the centrosome to the nucleus (Aylon et al., 2006). The findings of Aylon et al. indicate that ASPP1 accompanies

LATS2 under these conditions. Prior to such stimuli, LATS2 is present in the cytoplasm, in complex with ASPP1. Although the localization studies done thus far do not indicate an association of ASPP1 with the centrosome, the intimate association of ASPP1 and LATS2 in unstressed cells detected by coimmunoprecipitation suggest that such a possibility may exist. The precise role of LATS as a centrosomal protein and the potential impact that ASPP1 may have on such functions are also an area worth exploring.

It should be noted that these studies have not distinguished between the two LATS kinases nor do they address possible differences between YAP and the closely related TAZ protein. Mouse knockout studies clearly show distinct roles for ASPP1 and ASPP2 (see Sullivan and Lu, 2007). However, ASPP2 has also been implicated in p53-dependent transcription of target genes relevant to apoptosis (Samuels-Lev et al., 2001). The fact that the gene encoding LATS2 itself is a transcriptional target for p53 creating a positive feedback loop (Aylon et al., 2006) raises the question of whether p73 may have a similar role. All these added complexities need to be explored and certainly have created a cottage industry for this burgeoning field.

It should be emphasized that the two studies by Aylon et al. and Vigneron et al. provide an important new paradigm for understanding the functions of a cancer gene product. The same protein, in this case ASPP1, can exert distinct and apparently opposing effects depending upon its subcellular localization. Most importantly, the relative levels of each player in this complex pathway—ASPP1, YAP, and LATS—coordinately determine whether the outcome is oncogenic or tumor suppressing. Elucidating the key determinants for this will certainly provide fascinating science and serve as a basis for novel approaches to understand and treat human cancer.

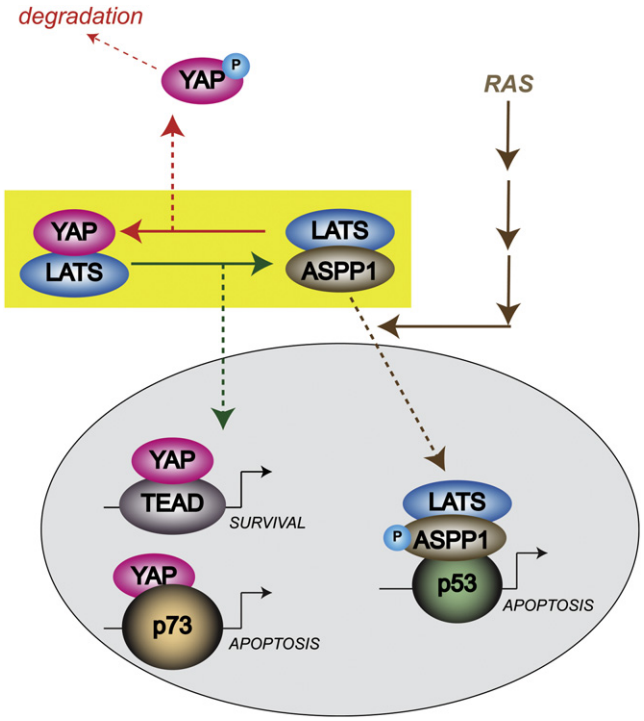

Figure 1. Mutually Exclusive Binding of ASPP1 or YAP to LATS Kinases Determines Their Subcellular Localization and Respective Roles in Regulating Gene Expression and Cell Survival

The YAP transcriptional cofactor and the ASPP1 protein bind to the LATS kinases (LATS1 and LATS2) in a mutually exclusive manner (Aylon et al., 2010; Vigneron et al., 2010). The binding of YAP to the LATS kinases leads to its phosphorylation. This results in cytoplasmic sequestration of YAP and its degradation via the proteasome (see Bertini et al., 2009). Cytoplasmic ASPP1 in unstressed cells interacts with the LATS kinases and prevents this YAP phosphorylation, leading to the nuclear accumulation of YAP (Vigneron et al., 2010). Nuclear YAP has been shown to mediate distinct gene expression responses (see Bertini et al., 2009). Interactions with the TEAD transcription factor enhances cell survival (Zhao et al., 2008), whereas binding to the p53 family member p73 promotes apoptosis (Strano et al., 2005). In cells transformed by activated Ras, the LATS kinases phosphorylate ASPP1, resulting in the translocation of the complex to the nucleus where it interacts with p53 and stimulates a gene expression profile that contributes to an apoptotic response (Aylon et al., 2010; Samuels-Lev et al., 2001). High levels of YAP, as found in subsets of human tumors, can, in turn, inhibit the interaction of LATS with ASPP1 and prevent this nuclear accumulation of ASPP1 (Aylon et al., 2010).

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