SUMO wrestling with Ras

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ABSTRACT
This review discusses our current understanding of the small ubiquitin-like modifier (SUMO) pathway and how it functionally intersects with Ras signaling in cancer. The Ras family of small GTPases are frequently mutated in cancer. The role of the SUMO pathway in cancer and in Ras signaling is currently not well understood. Recent studies have shown that the SUMO pathway can both regulate Ras/MAPK pathway activity directly and support Ras-driven oncogenesis through the regulation of proteins that are not direct Ras effectors. We recently discovered that in Ras mutant cancer cells, the SUMOylation status of a subset of proteins is altered and one such protein, KAP1, is required for Ras-driven transformation. A better understanding of the functional interaction between the SUMO and Ras pathways could lead to new insights into the mechanism of Ras-driven oncogenesis.

Ras genes are among the first oncogenes identified in human cancer and they are also among the most frequently mutated oncogenes. In mammals, the Ras family of small GTPases include 3 conserved members KRAS, NRAS and HRAS. Ras are membrane proteins and their activity is regulated through a GTP/GDP exchange cycle. Activation of receptor tyrosine kinases (RTKs) recruits Ras Guanine nucleotide exchange factors (GEFs) to the membrane which in turn promote Ras GTP loading. In addition to RTKs, Ras can also be activated by other signaling molecules including G protein-coupled receptors (GPCR), calcium influx and the T-cell receptor (TCR) complex. GTP binding leads to a conformational change in Ras and enables it to interact with a number of downstream effector proteins (Fig. 1). The effectors of Ras comprise of the MAP kinase (MAPK) pathway, the PI 3-kinase (PI3K) pathway, the small GTPases Rho, Rac, RaLA and RaLB, and the lipid enzyme phospholipase-C. These pathways coordinately regulate cell proliferation, survival, growth and motility. The role of Ras signaling in cancer has been recently reviewed. KRAS mutation occurs at a higher frequency than NRAS or HRAS, particularly in solid tumors. This might be due to different isoform-specific functions among Ras proteins as well as the ability of mutant KRAS to confer stem-like properties in cancer cells. Cancer cells that harbor Ras mutations often exhibit oncogene addiction to Ras, thus the Ras pathway represents a promising drug target in these cells. Direct inhibition of mutant Ras proteins, particularly KRAS, has proved difficult pharmacologically. However, currently there is a major effort underway to develop novel KRAS inhibitors based on new biochemical and structural insights of its function. Downstream of Ras, the MAPK pathway represents an attractive target as it is essential for Ras-dependent cell proliferation. However, inhibitors targeting the MAPK pathway, such as RAF and MEK kinase inhibitors, have not been particularly effective at shrinking Ras mutant tumors in patients. RAF inhibitors are unable to block MAPK signaling in Ras mutant cells due to their ability to paradoxically activate CRAF in this context. MEK inhibitors failed demonstrate substantial benefits in phase II trials among patients whose tumors were not genotyped for Ras mutation. In a recent study among lung cancer patients whose tumors harbor KRAS mutation, MEK inhibitor in combination with chemotherapy led to improved progression free survival but not overall survival. Thus, Ras mutant cancer remains a major therapeutic challenge. In addition to directly targeting Ras and its effector kinases, synthetic lethal and co-dependency studies have been employed.

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to explore additional genetic vulnerabilities in Ras mutant cancer cells beyond its canonical effectors.\textsuperscript{17} This approach is based on the idea that cancer cells driven by mutant Ras exhibit non-oncogene addiction to stress-response pathways for the maintenance of cell viability.\textsuperscript{18} Using this approach, we have recently identified the protein SUMO pathway as being required for Ras-driven transformation.\textsuperscript{19}

The SUMO pathway conjugates SUMO proteins onto lysine residues of target proteins (Fig. 1). Current knowledge of this pathway’s role in normal cellular function and in stress response has been reviewed recently.\textsuperscript{20-22} In mammals there are 3 SUMO proteins (SUMO1, 2 and 3). All three isoforms can form mono-conjugates, whereas SUMO2 and SUMO3 are highly homologous and can also form poly-SUMO chains. Thousands of cellular proteins have been shown as substrates for SUMOylation, thus this pathway could regulate a wide array of cellular processes. SUMOylation often occurs on lysine residues in the sequence context $\Psi$KxE/D, where $\Psi$ represents hydrophobic amino acids with large side chains and x represents any amino acids.\textsuperscript{20} In some instances SUMOylation can also occur on lysine residues not residing in the consensus motif.\textsuperscript{20} Analogous to protein ubiquitination, SUMOylation occurs in several distinct steps. First, the newly synthesized SUMO precursor protein is cleaved by sentrin-specific proteases (SENP$s$). SUMO is then activated by the E1 activating enzyme and transferred to the E2 enzyme. In the last step, E3 proteins promote the conjugation of SUMO to either a substrate protein or an existing poly-SUMO chain. SUMOylation is reversible via cleavage by SENPs. In mammalian cells...
there is a single E1 enzyme formed by the SAE1/SAE2 heterodimer, a single E2 enzyme Ubc9 and several E3s and SENPs. In contrast to the ubiquitin pathway where hundreds of E3s exist to serve different target proteins, the number of functionally validated SUMO E3s is small (less than 20). Thus, how substrate specificity is determined in the SUMO pathway remains an enigma.

The identification of SUMO substrates has been greatly facilitated by protein mass-spectrometry.\(^{23,24}\) For many proteins, SUMOylation serves to regulate their activity and/or subcellular localization rather than their stability. However, poly-SUMOylation can act as a recognition signal for SUMO-targeted ubiquitin ligases (STUBLs) that degrade poly-SUMOylated proteins.\(^{25}\) The majority of SUMOylation appears to occur in the nucleus and many transcription factors/co-factors, DNA binding proteins and chromatin-remodeling proteins are subject to SUMOylation.\(^{23,24}\) SUMOylation can either positively or negatively regulate the function of these proteins through mechanisms including changes in DNA-binding affinity, alterations in subcellular localization, induction or inhibition of protein-protein interaction, and modification of chromatin structure. SUMOylation of individual proteins can be regulated through proximity to the ligase and through phosphorylation (see below). With the exception of a few proteins including PML and RanGAP that show near stoichiometric levels of SUMOylation, most cellular proteins appear to be SUMOylated at low levels. There is evidence that the SUMOylated form of a protein could represent its active form, thus even low levels of SUMOylation could critically influence protein function. Indeed, the SUMO pathway is essential for cell viability: deletion of Ubc9 in mouse is lethal\(^{26}\) and cells without Ubc9 failed to complete mitosis.\(^{27}\)

How the SUMO pathway is itself regulated is not well understood. It is known that this pathway plays a critical role in cellular stress response. For example, transient heat shock induces a global increase in SUMOylation that serves to preserve cell viability.\(^{23}\) In the DNA damage response, SUMOylation is required for the proper localization of DNA repair proteins to sites of DNA damage and for their subsequent activation.\(^{22}\) SUMOylation affects protein complex formation in 2 ways. The addition of a bulky SUMO moiety could occlude a protein-binding domain and thus disrupt protein-protein interaction. On the other hand, many proteins possess short, hydrophobic SUMO-interaction motifs (SIMs) that bind to SUMO proteins with moderate affinity and specificity.\(^{20}\) It has been proposed that SUMOylation can serve as a “molecular glue” to hold protein complex together through extensive SUMO-SIM interactions.\(^{28}\)

The SUMO pathway’s role in cancer is rather complex. Genes in this pathway (E1, E2, E3 and SENPs) are not significantly mutated, amplified or deleted in cancer, thus they are unlikely to be oncogenes or tumor suppressors themselves. Expression of the SUMO E2 ligase Ubc9 is up-regulated in colorectal cancer and multiple myelomas, but down-regulated in advanced breast and lung adenocarcinomas.\(^{29,30}\) In prostate cancer, the expression of Ubc9 appears to be stage-dependent.\(^{30}\) Because SUMOylation is critical for stress protection, cell cycle and DNA repair,\(^{20,22,27,31}\) this pathway is likely to play a supportive role in tumorigenesis. This is consistent with the notion that this pathway is required for KRAS-driven transformation and for the viability of Myc-driven cancer cells.\(^{19,32}\) However, since SUMOylation of both oncoproteins and tumor suppressors has been described, the role of this pathway in cancer is likely to be determined in a context-dependent manner. Among SUMO substrates, several transcription factors with known roles in cancer are repressed by SUMOylation. These include Ets-1, c-Myb, androgen receptor, and MITF.\(^{33-36}\) In some cases, SUMOylation can promote transcription activation. For example, SUMOylation of the transcription co-factors TBL1 and TBLR1 release them from corepressor complex and enable them to bind β-catenin and promote the assembly of transcription activator complex.\(^{37}\)

A growing body of evidence indicates that the SUMO pathway functionally interacts with the Ras/MAPK pathway. Although human Ras proteins are only found to be ubiquitinated but not SUMOylated,\(^{38,39}\) the Drosophila Ras protein has been identified as a SUMO substrate.\(^{40}\) Downstream in the Ras/MAPK pathway, both MEK1 and MEK2 kinases are direct SUMO targets.\(^{41}\) SUMOylation of a conserved lysine residue on MEK1 and MEK2 leads to inhibition of their kinase activity. Interestingly, Ras oncoprotein, but not BRAF oncoprotein, can down regulate MEK SUMOylation due to Ras’ ability to inhibit the binding between MEK and its SUMO E3 ligase MEKK1.\(^{41}\) Further downstream of the Ras/MAPK pathway, the activity of the Ets-family transcription factor Elk-1 is inhibited by SUMOylation.\(^{42}\) Within the PI3K/Akt pathway, Akt1 has been reported as a direct target of SUMOylation, and this modification enhances its kinase activity.\(^{43,44}\) Whether other Ras effectors are subjected to regulation by SUMO is currently unknown.

Genetic studies also support a functional interaction between the SUMO pathway and Ras signaling. In C. elegans, mutations in SUMO pathway genes can modulate RTK-mediated Ras signaling during vulva development.\(^{35}\) The worm Elk-1 ortholog, Lin-1, is inhibited by SUMOylation.\(^{46}\) In Drosophila, SUMO knockdown inhibits ERK activation downstream of wild type (WT) Ras protein but not mutant Ras protein, indicating that SUMO modulates this pathway at or above the level of Ras.\(^{40}\) Through an
RNAi screen aimed at identify synthetic lethal partners of the KRAS oncogene in human colorectal cancer cell lines, we found that KRAS mutant cells are more sensitive to the depletion of the E1 ligase SAE1 and the E2 ligase Ubc9, particularly under anchorage-independent conditions.18,19 RNAi-mediated Ubc9 knockdown strongly inhibits the colony growth of cancer cells and KRAS-mediated transformation of immortalized normal epithelial cells. In colorectal cancer cells, inhibition of the SUMO pathway does not appear to affect MAPK signaling, thus the mechanism is likely to be indirect. Using mass-spectrometry to identify global changes in protein SUMOylation that is associated with KRAS mutation, we found that KRAS mutation does not affects global SUMOylation levels but instead alters the SUMOylation of only a small subset of proteins we termed KRAS-associated SUMOylated proteins (KASPs). We further showed that SUMOylation of one such KASP, the KRAB-associated protein 1 (KAP1/TRIM28), functionally contributed to KRAS-driven transformation. KAP1 is a transcriptional co-repressor protein with multiple functions.47 It associates with KRAB-domain zinc-finger proteins through its N-terminus,48 with HP1 through its HP1 binding domain,49-51 and with a number of chromatin-remodeling complexes including N-CoR, NuRD, and SETDB1 through its C-terminus.52-54 In addition to its transcriptional co-repressor activity, KAP1 serves several transcription-independent roles. KAP1 is a scaffold protein for DNA damage repair,55,56 a SUMO E3 ligase,57 and an ubiquitin E3 ligase.58,59 KAP1 is SUMOylated on several lysine residues near its C-terminus, and SUMO modification is required for its transcriptional co-repressor activity.57,60,61 We found that KAP1 is hyper-SUMOylated in KRAS mutant cells, particularly under anchorage-independent conditions, and the expression of a SUMO-KAP1 fusion protein could partially rescue Ubc9 depletion. Furthermore, we found that KAP1 knockdown inhibits the anchorage-independent growth of KRAS mutant cells, and this defect can be rescued by the expression of WT KAP1 but not a SUMO-deficient KAP1.19 KAP1 is not the only KASP that is required for KRAS-driven transformation since KAP1 over-expression can only partially rescue Ubc9 knockdown. It is likely that additional KASPs are also co-opted by the KRAS oncogene to support the growth and survival of cancer cells. The fact that only a small number of KASPs were identified in our mass-spectrometry analysis suggest that KRAS-dependent regulation of their SUMOylation is likely to occur through a specific, yet unknown mechanism that warrants further investigation. In addition, how KAP1 contributes to KRAS-driven transformation, and which of the many functions of KAP1 is relevant in this context remains to be elucidated.

One mechanism by which the Ras and SUMO pathways functionally interact is through the co-regulation of substrate proteins. It has been shown that the phosphorylation status of a protein can both positively and negatively influence its SUMOylation. For example, the ETS family transcription factor Elk-1 is SUMOylated under basal condition and this serves to repress its activity. Upon activation of the MAPK pathway, ERK phosphorylates Elk-1 and this inhibits Elk-1 SUMOylation and activates Elk-1.42 On the other hand, ERK-dependent phosphorylation of the nuclear body protein PML enhanced its SUMOylation in response to arsenic oxide treatment.62 These prior studies thus suggest the possibility that some of the KASPs uncovered in our study could be regulated by ERK phosphorylation in an analogous fashion. In some cases, regulation of SUMOylation by phosphorylation occurs through a phosphorylation-dependent SUMOylation motif (PDSM) within the amino acid sequence context KxExxS/T. In this scenario, SUMOylation on the lysine is directly controlled by the phosphorylation status of the adjacent serine/threonine residue.63,64 A PDSM of the sequence KxExxS/TP has the potential to be regulated by ERK. Bioinformatics analyses have discovered a number of transcription factors containing this PDSM.63,65,66 To date, however, few of these ERK-regulated PDSMs have been demonstrated experimentally. Nevertheless, this represents an attractive mechanism by which the Ras and SUMO pathways could functionally intersect. It would be interesting to determine if some of the KASPs we uncovered can be regulated through an ERK-directed PDSM.

The SUMO pathway presents a potential targeting opportunity in cancer. Significant efforts have been devoted toward targeting the ubiquitin pathway, and inhibitors of ubiquitin E1, E2, E3s and de-ubiquitnases have been developed.67 This prior experience could guide the development of SUMO pathway inhibitors. Several inhibitors of this pathway have been reported68-75 and the development of highly specific SUMO ligase inhibitors will be critical for evaluating the druggability of this pathway in cancer. Given the essentiality of this pathway, SUMO inhibitors could lead to significant off-target toxicity. However, the clinical success of proteasome inhibitors and the development of neddylation inhibitors67 suggest that inhibitors of the SUMO pathway could also hold potentials as anti-cancer agents.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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