REVIEW

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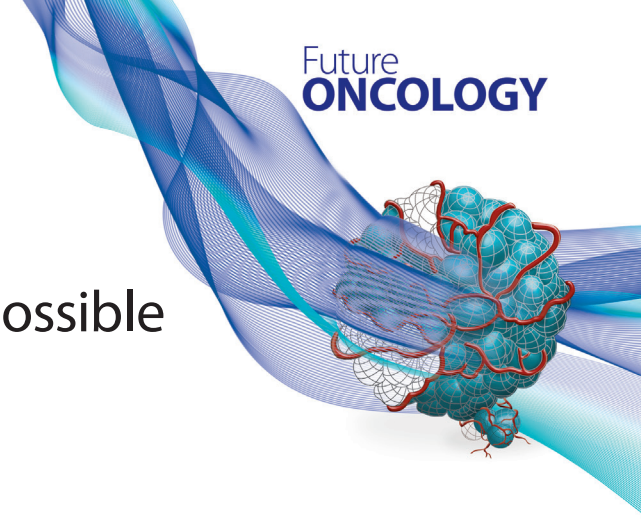

SUMO pathway components as possible cancer biomarkers

Domenico Mattoscio[1] & Susanna Chiocca*[,1]

ABSTRACT SUMOylation is a key post-translational modification that regulates crucial cellular functions and pathological processes. Recently, Small Ubiquitin-related MOdifier (SUMO) modification has emerged as a fundamental route that may drive different steps of human tumorigenesis. Indeed, alteration in expression or activity of one of the different SUMO pathway components may completely subvert cellular properties through fine-tuning modulation of protein(s) involved in carcinogenic pathways, leading to altered cell proliferation, apoptosis resistance and metastatic potential. Here we describe some of the most interesting findings pointing to a clear link between SUMO pathway and human malignancies. Importantly, a putative role for SUMO enzymes to predict cancer behavior can be speculated, and thus the possible application of alterations in SUMO pathway components as tumor biomarkers is discussed.

KEYWORDS
• biomarkers • cancer • SAE1/2 • SUMO • SUMO ligases • SUMO proteases • Ubc9

Small Ubiquitin-related MOdifier (SUMO) is a post-translational modifier belonging to the ubiquitin-like proteins family that plays key roles in regulating virtually all cellular functions [1]. Aberrant SUMOylation may thus have fundamental roles in the onset of human diseases, including cancer [2]. Indeed, the relationship between altered expression of SUMO system components and several kinds of cancer models is now clearly emerging.

The goal of this review is therefore to provide recent paradigmatic examples of human malignancies in which SUMO pathway components are abnormally expressed, underlying their contribution to cancer pathogenesis and, accordingly, the potential benefits of their use as cancer biomarker for risk assessment, disease progression, prognostic evaluation and therapeutic predictivity. We will first provide some basic concepts of protein SUMOylation, followed by a detailed description of the evidence highlighting a role for SUMO components in cancer progression.

The SUMO cycle

SUMOylation is a post-translational modification that allows the reversible attachment of one SUMO moiety to particular lysine residues of target proteins (see [3] for a recent review).

Firstly identified in 1996 by two independent labs [4,5], four different *SUMO* paralogs (1–4) have been described to date in the human genome, each encoded by distinct genes and, at least partially, destined to diverse functions on different targets [6]. SUMO2 and 3 are nearly identical in their primary sequence and are therefore collectively referred to as SUMO2/3 [7]. While SUMO1–3 are ubiquitously expressed in human tissues, *SUMO4* mRNA is expressed in kidney, lymph node and spleen [8], but is still unclear whether it can be processed and conjugated *in vivo* [9,10].

Similar to the ubiquitylation pathway, SUMO conjugation is catalyzed by a three-step enzymatic reaction **(Figure 1)** involving a dimeric SUMO activating enzyme E1 (named SAE1-SAE2 or

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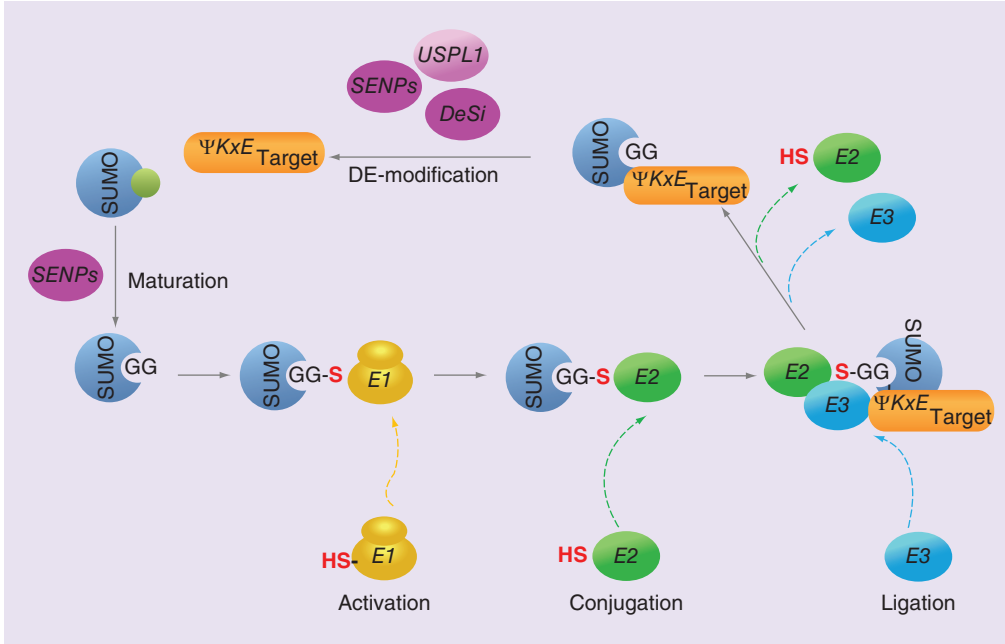

Figure 1. Schematic representation of the small ubiquitin-like modifier conjugation pathway.
SUMO precursors are processed by SUMO proteases (SENPs) that remove the C-terminal tetrapeptide (green sphere) and free the diglycine motif (maturation). The mature form of SUMO is then activated by the E1 enzyme SAE1/SAE2 (activation), and transferred on the E2 conjugating enzyme Ubc9 (conjugation). Afterward, SUMO is attached to specific lysine residue in the substrate often with the help of E3 ligases (ligation). Finally, SUMO proteins are removed from substrates by SUMO proteases cleavage (demodification), and free SUMO proteins are available for another conjugation cycle. See text for further details.
SENP: Sentrin-specific protease; SUMO: Small Ubiquitin-related MOdifier.
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Aos1-Uba2), one SUMO conjugating enzyme E2 (Ubc9), several SUMO ligases E3, and SUMO proteases required both to activate the SUMO precursor before its conjugation and to remove conjugated SUMO from its substrates. SUMOylation results in the formation of an isopeptide bond between the C-terminal glycine residue of the SUMO protein and the ψ-amino group of a lysine residue usually within the minimal consensus motif ψ-Lys-X(Asp/Glu) (where ψ is a large hydrophobic residue, X is any residue) in the acceptor protein [11]. Interestingly, SUMO may modify a single or multiple lysine residues on the same target protein, or can form SUMO chains on its substrates [12].

SUMOs are 11 kDa proteins initially synthesized as inactive precursors, which require an initial maturation step before their conjugation. This reaction, mediated by a specific protease belonging to the sentrin-specific proteases (SENPs) family [13], removes the carboxy-terminal proteolytic end of the immature SUMO protein to expose the diglycine motif before ATP-dependent activation by the SUMO E1 activating enzyme. SUMO E1 is a 110 kDa protein, composed of a heterodimer of SUMO-activating enzyme (SAE) 1 and 2 subunits that activates SUMO through the formation of a thioester bond between the C-terminal carboxy group of mature SUMO and the catalytic cysteine residue of SAE2 [14]. Once activated, SUMO from the E1~SUMO thioester is then transferred by a lateral trans-esterification reaction onto the conserved catalytic cysteine 93 of the E2 enzyme, Ubc9, generating an E2~SUMO thioester [15]. Although Ubc9 itself can directly interact and transfer SUMO on the consensus motif of several target proteins [16], specific SUMO E3 ligases are required to facilitate the modification usually using two different mechanisms: by promotion of SUMO discharging from Ubc9 to substrates or by correctly orientating the SUMO~E2 complex to enhance substrate specificity. A number of SUMO E3 ligases have been described and

classified accordingly to their similarity to ubiquitin E3 ligases. The members of the first group have an internal Siz/PIAS (SP)-RING domain, similar to the RING domain found in the ubiquitin E3 ligases [17], necessary for enhancing SUMO ligation efficiency. Human E3 ligases belonging to this group encompass members of the protein inhibitor of activated STAT (PIAS) family (PIAS1, PIAS3, PIASxα, PIASxβ and PIASy, reviewed in [18]) and Nse2/Mms21 [19]. Structural studies revealed that these E3 ligases promote SUMO modification by stabilizing the interaction of the target with the charged E2, bringing the E2~SUMO thioester in close proximity with the target lysine [20].

RanBP2, a long cytoplasmic fragment of the Nuclear Pore Complex, represents the only member of the second group of the SUMO E3 ligases. Differently from the SP-RING ligases described above, RanBP2 SUMO ligase activity resides in an Internal Repeat domain of about 100 residues (named R1-M-IR2) that associates with the SUMO-modified RanGAP1 and Ubc9. This ternary complex represents the functional E3 ligase multisubunit [21] that stimulates the E2 enzyme to discharge SUMO [22].

The third group comprises the polycomb member Pc2 (also known as CBX4) [23] that promotes SUMO conjugation of CtBP1 and a more limited repertoire of substrates as compared with other E3 ligases through two SUMO-interacting motifs. These motifs contribute to noncovalent SUMO binding of the E2~SUMO complex, allowing the proper localization of the active form of Ubc9 on substrates [24].

At least eight members of the diverse tripartite motif (TRIM) family have been recently described as a fourth group of SUMO E3 ligases, requiring both a RING domain and B-boxes (zinc-binding domains) to stimulate SUMO conjugation to target proteins [25,26]. Finally, several other proteins have been reported as E3 enzymes although the molecular details of how they promote SUMO modification remain less clear. This class includes proteins such as Topors, UHRF2 and TIF1γ, ubiquitin E3 ligases that also promote SUMO conjugation independently of their RING domain [27–29], while histone deacetylases 4 [30] and 7 [31], the G-protein Rhes [32] and TRAF7 [33] have also been reported as RING domain-lacking SUMO E3s.

SUMOylation is a reversible process, whose deconjugation is carried out by SUMO isopeptidase, cysteine protease enzymes that cleave the terminal glycine of SUMO from the substrate lysine. In addition to their role in selectively removing SUMO from target proteins, several SUMO proteases are also responsible for SUMO precursor maturation (see above). To date, three classes of SUMO proteases have been described in mammals, differing from each other in their cellular distribution, deconjugation specificity and SUMO maturation activities [34]. The largest and best-known family of proteases that catalyze SUMO processing and deconjugation includes six SENPs in humans (SENP1–3 and SENP5–7) (reviewed in [13]). SENP1 and SENP2 process and deconjugate both SUMO1 and SUMO2/3, while SENP3 and SENP5 act preferentially on SUMO2/3. Finally, SENP6 and SENP7 mainly deconjugate SUMO2/3 polymeric chain. Opposite to SENPs that localize in the nucleus or nucleus-associated structures, DeSI (-1 and -2) proteins are expressed also in the cytoplasm and show deSUMOylating, but not processing, activity for SUMO1 and SUMO2/3 in a very restricted number of substrates. Moreover, DeSI proteins also show weak ability in cleaving SUMO2/3 polymeric chains [35]. Finally, the last class of SUMO isopeptidases is represented by USPL1, a recently described broad SUMO deconjugating enzyme that is localized in Cajal bodies within the nucleus [36].

At the molecular level, the consequences of protein SUMOylation are target-specific but generally may fall in one of the three different following effects [37]. Firstly, SUMO attachment may mask binding sites of the substrate protein with its cellular interactors; conversely, the attached SUMO moiety may engender new docking sites for interacting proteins, usually through different SUMO interacting motifs [38]; finally, covalently bound SUMO can result in a conformational change of the modified target again affecting its interactors hub, enzymatic activity or cellular localization.

Through these simple mechanisms, SUMOylation can quickly regulate a number of cellular processes, such as transcriptional regulation, DNA repair, nucleocytoplasmic transport, cell signaling, mRNA maturation, meiosis, mitosis, chromatin remodeling, ion channel activity, cell growth and apoptosis [39]. Since SUMOylation clearly affects most cellular processes and functions, protein SUMOylation is expected to be important in a variety of diseases and, accordingly, altered in human cancer. Indeed, expression of SUMO components

has been extensively studied in various tumors. Therefore, in the next sections we will detail recent paradigmatic examples of human malignancies in which expression of the SUMO components has been shown to be altered (Table 1) and we will discuss their potential use as cancer biomarkers.

SUMO & cancer

• E1 enzyme

The importance of the SUMO activating enzyme in human malignancies comes from two recent studies suggesting that E1 may be an attractive target for the development of novel therapeutic approaches against cancer with specific genetic backgrounds. Indeed, a genome-wide shRNA library identified genes encoding the SUMO E1 subunits *SAE1* and *SAE2* among the genes with the strongest synthetic lethal interactions with K-Ras [40]. In fact, shRNAs against *SAE1* and *SAE2* selectively impaired the oncogenic K-Ras mutant, but not the nononcogenic K-Ras wild-type, colorectal cancer cells viability, suggesting that *SAE* genes are essential for K-Ras induced cancer growth. Therefore, we can speculate toward the use of SAE1/2 to evaluate the aggressiveness of mutated K-Ras-dependent malignancies.

Consistently, using a similar shRNA screen, the same authors also identified the SUMO E1 enzyme as fundamental in Myc-driven breast cancer tumorigenesis [41], since SAE2 was required to support Myc-dependent carcinogenesis both *in vitro* and in mice. Indeed, *SAE2* depletion in this genetic background led to reduced tumor growth of Myc-dependent breast cancer by triggering mitotic defects. In addition, breast cancer patients with high levels of Myc activation and lower SAE1 and SAE2 expression had significantly lower instances of metastatic cancer and increased survival compared with those with higher SAE1 and SAE2. Therefore, measurements of SAE1 and SAE2 expression could be a feasible tool to assess and predict the malignant potential in breast cancer patients.

• E2 enzyme

Opposite to the few studies pointing to a role of the E1 enzyme in human malignancies, the unique SUMO E2 Ubc9 is frequently upregulated in neoplastic tissues, and thus is a potential protein to approach for therapy [42] and as a cancer biomarker.

For instance, Ubc9 was found overexpressed in ovarian carcinoma cell lines and tissues where it substantially contributes to tumorigenesis by regulating *bcl-2* expression and apoptosis [100]. Additionally, increased Ubc9 levels were found in hepatocellular carcinoma as a consequence of its Cdc2-mediated phosphorylation that increases Ubc9 stability and expression [43], and in patients with acute myeloid leukemia (AML) carrying the C/EBPα mutation. In this last clinical setting, Ubc9 accounted for the myeloid-differentiation block by linking mutated and wild-type C/EBPα activities. Indeed, mutated C/EBPα activates Ubc9 transcription and, in turn, Ubc9 itself promotes wild-type C/EBPα SUMOylation and its transcriptional repression responsible for the myeloid-differentiation block [45]. Therefore, the evaluation of Ubc9 expression could be helpful in the diagnosis of ovarian and hepatocellular carcinoma, and in particular in AML patients.

Moreover, tissue microarray analysis found that Ubc9 expression increases during progression from normal colonic epithelium to early and advanced stages of colon cancer [46]. Similarly, Ubc9 was upregulated in prostatic intraepithelial neoplasia and its expression was even higher in primary prostate adenocarcinoma, suggesting again its use also as prostatic progression marker. On the contrary, in metastatic tissues derived from breast, prostate and lung cancer, Ubc9 expression was decreased in comparison with their corresponding normal tissues and primary tumors, suggesting a specific regulation of its expression during tumor evolution [46]. Finally, Ubc9 detection by antibody arrays revealed increased Ubc9 expression in tissue biopsies taken from later stages of the melanoma progression pathway, and in melanoma-infiltrated lymph nodes, pointing to an important role for Ubc9 during melanoma progression and metastasis. Indeed, Ubc9 showed a protective role against apoptosis induced by chemotherapeutic drugs in melanoma cells [47]. Altogether, these findings suggest the use of Ubc9 as a biomarker to follow the progression of colon, prostatic and melanoma cancers.

In addition, in lung cancer tissues, higher Ubc9 expression [48,49] correlated with advanced disease as compared with lower Ubc9-expressing lung cancer patients, which instead were associated with an increased survival [50]. Furthermore, the *UBC9* 10920CG genotype can be used to predict response rate to irinotecan in lung cancer

Table 1. SUMO proteins and their putative role as cancer biomarkers in human malignancies.

SUMO component	Tumors	Proposed biomarker	Ref.
SAE1/SAE2	Colorectal	Prognosis	[40]
	Breast	Prognosis	[41]
Ubc9	Ovarian	Diagnosis	[42]
	Hepatocellular carcinoma	Diagnosis	[43,44]
	AML	Diagnosis	[45]
	Colon	Progression	[46]
	Prostate	Progression	[46]
	Melanoma	Progression	[47]
	Lung	Prognosis, therapeutic predictivity	[48–51]
	Squamous cell carcinoma of the head and neck	Diagnosis, prognosis	[52]
	Glioma	Diagnosis, prognosis	[53,54]
	Breast	Diagnosis, prognosis, therapeutic predictivity, risk assessment	[44,49,55–59]
	Astrocytic brain	Diagnosis, progression	[60]
PIAS1	Colon	Diagnosis, prognosis	[61]
	Gastric	Diagnosis, prognosis, therapeutic predictivity	[62,63]
	Prostate	Diagnosis	[64]
	Breast	Prognosis	[44]
PIAS3	Lung, breast, prostate, colorectal, brain	Diagnosis	[65,66]
	Ovarian, endometrial	Diagnosis	[67]
	Squamous cell carcinoma of the lung	Diagnosis	[68]
	Gastric	Diagnosis, prognosis	[69]
PIAS4	Gastric	Therapeutic predictivity	[63]
	Breast	Prognosis	[70]
	MDS	Diagnosis, progression	[71]
RanBP2	Multiple myeloma	Diagnosis	[72]
	Small cell lung cancer	Diagnosis	[73]
	Inflammatory myofibroblastic tumor	Diagnosis, prognosis, therapeutic predictivity, risk assessment	[74,75]
	Acute myelomonocytic leukemia	Diagnosis, risk assessment	[76,77]
	8p11 myeloproliferative syndrome	Diagnosis, risk assessment	[78]
Pc2	Hepatocellular carcinoma	Prognosis	[79]
TRIM19	AML	Diagnosis	[80]
TRIM27	Endometrial	Prognosis	[81]
	Colon	Prognosis	[82]
	Ovarian	Prognosis, therapeutic predictivity	[83]
SENP1	Prostate	Diagnosis, prognosis, therapeutic predictivity	[84–86]
	Pancreatic ductal adenocarcinoma	Diagnosis, prognosis	[87]
SENP2	Bladder	Diagnosis	[88]
	Hepatocellular carcinoma	Diagnosis	[89]
SENP3	Colon, rectum, ovarian, lung, oral squamous cell carcinoma	Diagnosis	[90,91]
SENP5	Oral squamous cell carcinoma	Diagnosis	[92]
	Breast	Prognosis	[93]
SENP6	Breast	Diagnosis	[94]
SENP7	Breast	Diagnosis	[95]
USPL1	Breast	Risk assessment	[96]
SUMO1, SUMO2/3	Astrocytic brain	Diagnosis, progression	[60]
SUMO1	Hepatocellular carcinoma	Diagnosis	[97]
	Lip	Diagnosis	[98]
	Oral squamous cell carcinoma	Diagnosis, prognosis	[99]

AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome.

chemotherapy, since associated with a better drug response than the C/C genotype [51]. A similar correlation between higher Ubc9 expression and disease severity has been also found in squamous cell carcinoma of the head and neck [52], and in glioma [53,54]. In particular, the combination of miR-214 downregulation and *UBC9* mRNA upregulation were significantly associated with more aggressive clinicopathological features and poorest overall survival of glioma patients, probably due to enhanced proliferation and apoptosis resistance conferred by Ubc9 itself [53].

Similarly to what was found in glioma, Ubc9 protein is also highly expressed in several breast cancer cells lines and tissues [49,55] partially due to miRNAs defects (miR-214 and miR-30e, in glioma and breast cancer cells, respectively [49,53]). Notably, enhanced Ubc9 expression could have a clear role in breast tumors by its SUMOylation-independent activities. Indeed, Ubc9 enhanced tumor growth [101], invasion and metastasis [102], by regulating *BLC-2* and miR-224 expression, respectively. In addition, Ubc9 is also a key determinant for breast cancer tumorigenesis by directly regulating estrogen receptor-α activity [55,103]. Therefore, the relevance of Ubc9 as a breast cancer marker relies on very strong biological evidence. Indeed, high Ubc9 expression is associated with higher tumor grade, larger tumor size, lymph node metastasis, poor clinical outcome and survival, and, importantly, with poor clinical response to chemotherapy [56,57]. Remarkably, not only the expression levels but also the Ubc9 intracellular localization can be used to evaluate breast cancer aggressiveness. Indeed, tumors with positive nuclear and negative cytoplasmic Ubc9 staining in immunohistochemistry (IHC) showed good prognostic features as compared with cytoplasmic positive-Ubc9 breast cancer [44]. Genetic variability may also affect Ubc9 expression and activity and may have an impact on breast cancer occurrence and progression. To this end, association between *UBC9* genotypes and histopathological parameters revealed an increased risk of breast cancer occurrence associated with different single nucleotide polymorphism (SNP) [58–59,104], implying the value of genetic Ubc9 variation for breast cancer risk assessment and prognosis.

Therefore, Ubc9 mRNA or protein expression, localization and SNPs may serve as useful biomarkers for diagnosis, prognosis, evaluation of therapeutic response and risk assessment in several kinds of human malignancies.

• E3 enzymes

Most of the current knowledge on the role of SUMO E3 enzymes and cancer mainly come from studies on PIAS1. Opposite to Ubc9 that is frequently upregulated in several primary human malignancies, suggesting that a boost in the SUMO pathway may confer selective advantage to tumor cells, the expression pattern of the E3 enzymes is more controversial. Indeed, PIAS1 protein levels in advanced tumor stages can be both downregulated or upregulated depending on the anatomic site of cancer growth. For example, lower PIAS1 expression has been reported in colon [61] and in gastric cancer [62], where its assessment by IHC could be helpful not only as a marker for preclinical detection, but also for the clinical valuation of patients with these tumors. Interestingly, PIAS1 restoration in human gastric cancer cell lines inhibited cell proliferation and invasion by targeting proteins of the MAPK pathway [62]. Consistently, gastric cancer patients with high *PIAS1* and *PIAS4* mRNA levels were also associated with an improved outcome upon docetaxel-based treatment, again pointing to a protective role of PIAS proteins toward tumor development and resistance to treatments [63]. However, PIAS1 may also promote tumorigenesis, for example in prostate cancer, where PIAS1 is overexpressed in malignant tissues. In this clinical setting, PIAS1 overexpression promoted, rather than inhibited, cell proliferation by altering cell cycle progression through alterations of p21 levels [64]. In addition, similarly to what observed for Ubc9, in breast cancer patients PIAS1 cytoplasmic localization was associated with a more aggressive tumor phenotype [44].

PIAS3 has also been found both overexpressed or downexpressed in different human malignancies. For example, increased PIAS3 was observed by IHC screening in a variety of human cancers, including lung, breast, prostate, brain and colorectal cancers, where it may be used as a useful molecular marker [65,66]. On the contrary, PIAS3 downregulation can be used as a valuable tool in ovarian and endometrial cancers [67], in squamous cell carcinoma of the lung [68], and in gastric carcinoma where the decreased expression levels of PIAS3 protein and mRNA in cancerous tissues were closely related to large tumor size and poor differentiation [69].

In addition, PIAS4 protein expression showed a significant correlation with higher histological

grades, and as a marker to predict poor outcome in triple-negative breast cancer [70].

DNA microarray analysis of stage progression in myelodysplastic syndrome (MDS) revealed PIAS4 physiologically expressed in AC133-positive hematopoietic stem cells both from healthy subjects and from patients in latent stages of MDS, but suppressed upon transition to the advanced stages of MDS where PIAS4 depletion facilitates cell growth [71]. Therefore, in this particular pathological context, PIAS4 expression discriminates among the different stages of MDS progression.

The expression level of RanBP2, the unique member of the second SUMO E3 ligases class, has been found increased in multiple myeloma [72] and in small cell lung cancer [73] both at mRNAs and at protein levels. Therefore, RanBP2 could be used as potential new biomarkers for the identification of these malignancies. However, rather than the RanBP2 amount, point mutations and *RANBP2* gene translocation are mostly involved in tumorigenesis. Indeed, *RANBP2* single point mutations have been found in some human colorectal cancers [105].

Moreover, *RANBP2* has been also recognized as one of the fusion partners of the *ALK* gene, expressed as a fusion chimera in several tumors [106]. In particular, *RANBP2–ALK* fusion has been reported in several cases of neoplasms such as inflammatory myofibroblastic tumor [74], where *RANBP2-ALK* fusion was associated with poor prognosis and a better response to the ALK inhibitor crizotinib [75], and in subtypes of AML [76,77]. Consistently, *RANBP2* was found as a novel fusion partner gene for *FGFR1*, confirming the relevance of *RANBP2* in myeloid neoplasms [78]. *RANBP2* fusion chimeras may thus be useful in the identification of these rare malignancies and to set out better clinical approaches.

The use of the SUMO E3 ligase Pc2 as a prognostic biomarker has been proposed only for hepatocellular carcinoma, where its overexpression induces cell proliferation. Indeed, Pc2 immunostaining in tumor cells was much stronger than in nontumor liver tissue and its high cytoplasmic levels correlated with poor prognosis in hepatocellular carcinoma patients [79].

Finally, the role of TRIM proteins in carcinogenesis has been studied in detail. We suggest that readers consult this recent excellent review [107]. Within the TRIMs that promote SUMOylation [26], the best known is promyelocytic leukemia (PML, also known as TRIM19), involved in the PML-RARα translocation specific of acute promyelocytic leukemia [80], while positive TRIM27 (RFP) expression is a predictive marker for an unfavorable clinical outcome in endometrial [81], colon [82] and ovarian cancer where its positivity significantly correlated with drug resistance [83].

SUMO proteases

The role of SENP1 in cancer progression and as putative biomarker has been deeply investigated. As an illustrative example, SENP1 may be used as a diagnostic and prognostic marker for prostate cancer, and to predict therapeutic outcome since it is overexpressed both in precancerous lesions and in cancer tissues [84], and its increased expression has been found to positively correlate with a more aggressive disease. Indeed, *in vitro* and *in vivo* experiments showed that SENP1 promotes colony formation, migration and invasion of prostate cancer cell lines, and tumor growth in mice [85]. In particular, the ability to trigger bone metastasis and secondary tumors could be at least partially due to a SENP1-mediated regulation of two bone-remodelling proteins, MMP2 and MMP9 [85]. SENP1-positive patients undergoing radical prostatectomy expression were significantly associated with poor biochemical-free survival [86]. SENP1 was also upregulated in pancreatic ductal adenocarcinoma tissues compared with adjacent normal tissues, and its expression was positively associated with lymph node metastasis and tumor aggressiveness [87].

Opposite to the frequent SENP1 contribution in malignant progression, SENP2 downregulation plays a key role in the onset and progression of bladder cancer [88] and hepatocellular carcinoma [89], suggesting its use as a biomarker.

Similarly to SENP1, SENP3 is elevated in prostate cancer, and also in additional tumors, including colon, rectum, ovarian and lung as demonstrated by tissue chip IHC [90], and oral squamous cell carcinoma [91], where also SENP5 expression is significantly higher in cancer tissues [92]. Furthermore, using bioinformatics analysis of published microarray data, SENP5 expression levels stratified breast cancer patients into two survival groups: those with low *SENP5* were associated with better prognosis and higher survival [93] compared with the high-expressing

SENP5 patients. Mechanistically, SENP5 promoted proliferation, migration and invasion of breast cancer cells probably by modulating the TGF-βRI/MMP9 axis. Using a similar approach, a recent report showed downregulation of *SENP6* mRNA in breast tumor tissues compared with normal tissues [94].

A very peculiar use of SENP7 as a breast cancer biomarker may emerge from a recent elegant study. Indeed, two *SENP7* transcripts exhibit inverse expression in breast cells. The shorter splice variant, *SENP7S*, is highly expressed in normal breast tissues, whereas the full-length *SENP7L* is increased in breast cancer patients. The long *SENP7* transcript deSUMOylate and repress the transactivation ability of the transcription factor HP1α, favoring cellular proliferation promoted by the altered gene-expression profiles [95]. Therefore, screening the amount of different *SENP7* transcripts may help in the identification of breast cancer tissues.

Finally, a large genotype analysis identified the SNP rs7984952 in *USPL1* gene correlated with risk for breast tumor development. Indeed, C allele homozygosis is associated with a lower risk of breast tumors with respect to the TT homozygotes, probably because the C allele was associated with increased *USPL1* expression [96].

SUMO proteins

In addition to the single SUMO pathway components, SUMO paralog aberrancies have been extensively studied in various tumors and therefore may be also used as putative biomarkers for certain types of human malignancies. Furthermore, a number of different carcinogenic proteins are SUMO-modified, and their number is still growing (see [108] for a recent review). Therefore, their SUMOylation status could be of clinical relevance.

More than the above described literature, where the alteration of one of the SUMO enzymes may also lead to specific or overall alterations in SUMO conjugation levels, other reports directly link SUMO paralogs with cancers. For instance, levels of both SUMO1 and SUMO2/3 conjugated proteins, together with Ubc9, were markedly increased during astrocytic brain tumors development. Indeed, SUMO1 and SUMO2/3 levels were higher in low-grade astrocytoma and even more in glioblastoma multiforme brain tumors, which carry a very poor prognosis [60]. Consistently, SUMO1 overexpression was detected in hepatocellular carcinoma patients [97], and in lip cancer [98] suggesting that the expression level of SUMO1 may aid their diagnosis. Moreover, SUMO1 was expressed at much higher levels in oral squamous cell carcinoma tissue than normal oral epithelium, and patients with SUMO1 and Mdm2 overexpression experienced more frequently local recurrences after initial treatments [99]. Therefore, SUMO1 may be useful as a diagnostic biomarker and clinical indicator for tumor recurrences together with Mdm2.

Conclusion

The results of the studies here described clearly underline that SUMOylation is a fundamental post-translational modification that not only crucially regulates cellular activities, but also has an important role in pathological processes as well. In particular, a role for SUMO enzymes in routes driving tumor formation is now widely accepted and supported by a plethora of evidence. Indeed, SUMOylation is in close relationship with cancer development, progression and metastasis, through molecular mechanisms that are still poorly understood. Significantly, genetic status, expression and activities of SUMO proteins were able to identify potential individuals at risk, to point out malignant tissues, to recognize different steps of cancer progression and to predict disease outcome and response to treatments. Therefore, we speculate that SUMO proteins may be used as potential biomarker in several human malignancies. In this respect, we also recently proposed Ubc9 as a marker to monitor the progression of human papillomavirus oncogenic infections in cervical tissues [109].

Future perspective

Although the potential benefits of SUMO components as cancer biomarkers are quite clear, much work needs to still be done for validation of their use in large, independent cohort of patients, to prove their clinical utility, and to set up standardized and automatized assays so that their possible usefulness may be reflected in routine clinical practices.

In addition, a better comprehension of the upstream signals that regulate expression and activity of SUMO enzymes is strongly required, since it will provide useful information on SUMO physiology and suggest novel

approaches to manipulate SUMOylation in order to combat human diseases. Finally, the activity of numerous key regulatory proteins are controlled by SUMO modification, and therefore specific or global changes in SUMOylation status driven by SUMO enzymes deregulation may completely subvert protein functions. Increasing numbers of studies have identified, and will keep identifying in the next few years, novel proteins involved in human malignancies whose activities are in tight conjunction with SUMO, and whose SUMOylation status might predict the state of the disease and provide useful information for cancer diagnosis, progression or prognosis.

We believe that more studies in the near future are required to understand the 'SUMO code' during tumor development, to predict cancer behavior and to provide multiple alternatives to fight it.

Financial & competing interests disclosure

Work related to the topic of this review in our laboratory is supported by grants from European Commission-FP7-HPVAHEAD and Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.) to S Chiocca. D Mattoscio is a Fondazione Italiana per la Ricerca sul Cancro (F.I.R.C.) fellow. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

The SUMO pathway

- Ubiquitin-like modification system catalyzed by the sequential action of E1, E2, E3 and SUMO proteases enzymes.
- SUMO1–3, and SUMO4 are the SUMO isoforms in mammals.
- E1 (SAE1/SAE2) activates SUMO.
- E2 (Ubc9) conjugates SUMO to target proteins.
- E3s (protein inhibitor of activated STAT (PIASs), RanBP2, Pc2, TRIMs) facilitate SUMO attachment to target proteins.
- SUMO proteases (sentrin-specific proteases, DeSis, USPL1): promote SUMO maturation and SUMO detachment from substrates.

SUMO & cancer

- E1 enzymes involvement in Ras- and Myc-driven tumors.
- Ubc9 overexpression promotes diverse human malignancies. Ubc9 as putative biomarker of ovarian, hepatocellular carcinoma, acute myeloid leukemia, colon, prostate, melanoma, lung, squamous cell carcinoma of the head and neck, glioma, breast, and astrocytic brain tumors. *UBC9* single nucleotide polymorphisms predictive of breast cancer.
- E3 ligases may act both as tumor promoters or tumor suppressors. PIAS1, PIAS3, PIAS4, RanBP2, Pc2, TRIMs levels may be related and used to identify different malignancies. *RANBP2* chromosomal translocation as a signature for several myeloproliferative disorders.
- Sentrin-specific proteases as a useful tool to evaluate prostate, pancreatic ductal adenocarcinoma, bladder, hepatocellular carcinoma, colon, rectum, ovarian, lung, oral squamous cell carcinoma and breast cancers. *USPL1* single nucleotide polymorphism may be able to predict breast cancer risk.
- SUMO1 and 2/3 expression to follow astrocytic brain tumor progression. SUMO1 expression may be able to identify hepatocellular carcinoma, lip and oral squamous cell carcinoma cancerous tissues.

Conclusion

- Proteins involved in the SUMO pathways play a fundamental role in human carcinogenesis.
- The analysis of SUMO component as well as SUMOylated proteins can provide useful information on cancer behavior.
- SUMO proteins and their genes may be used to monitor different human malignancies.
- More work is still needed to validate SUMO alteration as cancer biomarkers.

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