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Invited review

Pharmacological treats for SUMO addicts

Marco P. Licciardello[a], Stefan Kubicek[a,b,*]

[a] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Lazarettgasse 14, 1090 Vienna, Austria
[b] Christian Doppler Laboratory for Chemical Epigenetics and Antiinfectives, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

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ABSTRACT

Non-oncogene addiction exploits cancer vulnerabilities resulting from altered cellular signaling pathways in response to oncogenic mutations that are not directly druggable. In this perspective, we address recent findings showing how the SUMOylation cascade provides a synthetic lethal target in the context of different malignant transformations. Functional genomics screens have revealed that the activation of oncogenes such as *NOTCH1*, *MYC* or *KRAS* generates a cancer-specific dependency on SUMOylation. Pharmacological targeting of the SUMOylation cascade induces cancer cell death in these settings, suggesting potential therapeutic applications in oncology. However, the physicochemical properties of the few currently available SUMOylation inhibitors preclude clear-cut investigations and clinical testing. We therefore encourage the development of better chemical probes targeting this multifaceted post-translational modification. Such optimized molecules would enable proof of concept studies to evaluate the therapeutic potential of non-oncogene addiction to SUMO.

Contents

1. Introduction

Malignant cells secure their growth advantage through the inactivation or downregulation of tumor suppressors and the activation or overexpression of oncogenes. It has been shown that tumors tend to become addicted to such alterations [1] and these observations have fostered the development of the first targeted anticancer therapies [2]: monoclonal antibodies and small molecule inhibitors have been engineered to target specific cancer-related proteins and show remarkable results in the clinic [3,4]. However, many cancer-causing genes have proved hard to target [5,6] and restoring the function of inactivated tumor suppressors remains challenging. Thus, alternative and more personalized pharmacological treatments are required.

The accumulation of genetic lesions rewires the circuitry of a cell generating novel connections in the molecular network. To cope with such changes tumor cells may develop specific dependencies on genes and pathways that are not directly responsible for the malignant transformation. This concept of "non-oncogene addiction" (also referred to as synthetic lethality) [7,8] has opened an alternative way to treat cancer and provided patient-tailored therapy that has already reached the clinic [9]. In this perspective, we illustrate how the SUMOylation cascade may be exploited as a synthetic lethal partner in the context of different activated oncogenes for the development of personalized anticancer treatments.

* Corresponding author at: CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Lazarettgasse 14 AKH BT 25.3, 1090 Vienna, Austria. Fax: +43 1 40160 970 000.
E-mail address: skubicek@cemm.oeaw.ac.at (S. Kubicek).

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2. The SUMOylation cascade

Protein function can be regulated and fine-tuned by post-translational modifications. The small ubiquitin-like modifier (SUMO) proteins are a class of ubiquitin-like proteins (ULPs) covalently attached to other polypeptides to modulate their activity, stability, cellular localization, and interaction partners [10]. Similar to other ULPs, SUMO travels along a cascade of enzymatic reactions to reach its final target but in contrast to ubiquitin, this post-translational modification does not normally trigger proteasomal degradation of proteins.

The human genome encodes four distinct isoforms of SUMO; SUMO1, SUMO2, and SUMO3 are ubiquitously expressed and share only specific targets. SUMO2 and SUMO3 are 97% identical and cannot be distinguished by currently available antibodies. The role of SUMO4, whose expression is restricted to kidney, spleen and lymph nodes, is still unclear. All SUMO isoforms are expressed as immature proteins activated by sentrin-specific proteases (SENP) [11]. These enzymes cleave a short stretch (9–11 amino acids) exposing a C-terminal Gly–Gly motif in the mature SUMO. At the beginning of the cascade, mature SUMO is first loaded on the heterodimeric E1 activating complex SAE1/UBA2. By means of ATP hydrolysis, the diglycine motif at the C-terminus of SUMO is conjugated via thioester bond formation to a cysteine residue in the active site of the UBA2 subunit. SUMO is then transferred to the E2 conjugating enzyme UBC9 (encoded by the *UBE2I* gene) forming again a thioester bond on a conserved cysteine. UBC9 escorts SUMO on its way to the final target, a step that might be assisted by E3 ligases such as the PIAS proteins [12]. Finally, target SUMOylation can be reverted by SENP proteases or deSUMOylating isopeptidases (DeSI-1, DeSI-2, and USPL1) [13,14] replenishing the pool of unconjugated mature SUMO (Fig. 1a). SUMOylation occurs on target protein lysines often within a Ψ-K-*x*-E/D sequence (where Ψ is a bulky aliphatic amino acid and *x* is any residue) [15]. Variations to this consensus site have been described [16,17] and SUMOylation events occurring on lysines outside of this motif have also been reported [18,19]. Even though some targets are quantitatively SUMOylated, either constitutively or in response to specific stimuli, most proteins show low steady-state levels of modification by SUMO [10]. Moreover, similar to ubiquitin, target proteins can be both monoSUMOylated and adorned with chains of SUMO [20,21]. The physiological role of SUMO chains remains, however, to be elucidated.

Protein post-translational modification by SUMO occurs both in the nucleus and the cytoplasm [22]. Indeed, SUMOylated proteins have been shown to be involved in a variety of cellular processes such as regulation of transcription, DNA repair, cell cycle and nuclear transport [23–27]. Such a widespread functionality designates the SUMOylation cascade as a hub enriched for synthetic lethal partners that might be exploited for the development of anticancer treatments [28].

3. Cancer non-oncogene addiction to SUMO

Non-oncogene addictions cannot be inferred from the analysis of cancer genomes and rather have to be investigated by means of chemical or genetic screens using appropriate models. In an attempt to discover novel personalized treatments for breast cancer we have recently performed a loss-of function screen on a panel of isogenic cells [29]. In contrast to genetically more complex cancer cell lines, isogenic systems allow for easier mechanistic interpretations of gene–gene or drug–gene interactions [30]. Moreover, they permit studies on cancer genes for which cell lines are not immediately available. We overexpressed 10 breast cancer oncogenes in MCF10A cells creating a panel of 10 isogenic systems modeling the activation of specific cancer-related genes in the mammary gland. On this panel, we have screened an epigenome-focused shRNA library and found breast epithelial cells overexpressing the intracellular domain of NOTCH1 (NOTCH1 cells) to be specifically sensitive to the knockdown of *UBE2I*, the gene encoding the E2 conjugation enzyme UBC9 of the SUMOylation cascade [29]. Signaling via the NOTCH1 transmembrane receptor regulates cellular proliferation and differentiation and has been found to be activated in different types of cancer [31–33]. The interaction with a ligand induces cleavage of the receptor by γ-secretase so that the intracellular domain of NOTCH1 may translocate to the nucleus and drive the transcription of canonical target genes such as *HES1*, *HEY1*, and *MYC*. Currently, pharmacological intervention on the NOTCH1 signaling pathway relies on monoclonal antibodies directed against the receptor or small molecule γ-secretase inhibitors (GSIs) [34]. These are being evaluated in the clinic but have already showed significant side effects [35]. Moreover, GSI-insensitive activation of NOTCH signaling has also been observed in breast cancer [36].

Overexpression of the potent NOTCH1 oncogene confers a growth advantage over the precursor MCF10A line, which is readily outcompeted by NOTCH1 cells in co-cultures. However, we have shown that NOTCH1 cells do not prevail anymore upon knockdown of *UBE2I* while rescuing of UBC9 by means of overexpression restored their growth advantage [29]. This finding discloses the SUMOylation cascade as a synthetic lethal partner of NOTCH1 signaling activation in breast cancer and indicates an alternative therapeutic approach for a specific tumor genotype. As we were interested in the pharmacological exploitation of this genetic interaction, we evaluated the sensitivity of NOTCH1 and MCF10A cells to ginkgolic acid (GA), a small molecule reported to inhibit SUMOylation through direct interaction with the SAE1/UBA2 heterodimer [37]. NOTCH1 cells showed greater sensitivity to GA compared to MCF10A cells. In addition, an alternative GSI-sensitive model of NOTCH1 activation (NOTCH1 ΔE cells) similarly responded to the small molecule inhibitor in a NOTCH-dependent fashion. Inhibition of the SUMOylation cascade initially slows the growth of our isogenic NOTCH1 model eventually leading to apoptosis [29]. A closer inspection of the cell cycle revealed impairments in the S and G2/M phases upon treatment of NOTCH1 cells with GA. Proteins involved in DNA replication and mitotic division have been reported to be post-translationally regulated by SUMO [25,38], thus providing an explanation for the observed phenotype.

Among the most striking evidence of the greater sensitivity of NOTCH1 cells to GA was the dramatic reduction of global protein SUMOylation assessed by SUMO1- and SUMO2/3-specific antibodies at GA concentrations where global SUMOylation levels were virtually unaffected in MCF10A cells [29]. These results suggest a stronger dependency of NOTCH1 cells on SUMOylation while the non-tumorigenic precursor line copes with the residual activity of the cascade in the presence of GA. Interestingly, both NOTCH1 cells and NOTCH1 ΔE cells showed the greatest depletion of unconjugated SUMO1 and SUMO2/3 among all the isogenic systems of our panel. We therefore hypothesized that activation of NOTCH1 signaling in breast epithelial cells would deplete unconjugated SUMO conferring sensitivity to further perturbations of the cascade (Fig. 1b). Indeed, downregulation of SUMO increased the sensitivity of parental MCF10A cells to GA while overexpression rescued NOTCH1 cells from the treatment with the inhibitor supporting our conclusion [29].

The isogenic cells employed in our study allowed an easier mechanistic dissection of the interaction that scored in the initial screen. However, our models cannot entirely represent the genetic complexity of real tumor samples. To validate our findings in a more clinically relevant context we administered GA to patient-derived NOTCH-activated breast cancer cell lines. Notably, the sensitivity of these cancer cells to inhibition of SUMOylation correlated to