

# Regulation of cancer stem-like cell differentiation by Smac mimetics

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**Keywords:** cancer stem cells, differentiation, IAP proteins, Smac mimetic

**Abbreviations:** CSLCs, cancer stem-like cells; GBM, glioblastoma; GFAP, glial fibrillary acidic protein; IAP, inhibitor of apoptosis; NF- $\kappa$ B, nuclear factor kappa B.

Small-molecule antagonists of inhibitor of apoptosis (IAP) proteins such as Smac mimetics are considered promising cancer therapeutics through the engagement of cell death pathways. Recent evidence suggests that Smac mimetics perform additional nonapoptotic functions by initiating differentiation in cancer stem-like cells, opening new perspectives for their future clinical application.

Glioblastoma multiforme (GBM), the most common primary malignant brain tumor in adults, is known for its high aggressiveness and inherent resistance to current treatment regimens.<sup>1</sup> These characteristics highlight the urgent need for new therapeutic concepts for GBM. Cancer stem-like cells (CSLCs) are typically a small subpopulation of tumor cells that are considered to sustain long-term clonogenic survival and tumor growth and confer treatment resistance.<sup>2</sup> In addition, CSLCs have been implicated in mediating resistance of GBM to chemotherapy or irradiation,<sup>2</sup> making them attractive targets for therapeutic intervention. Recent studies suggest that strategies aiming to stimulate differentiation of GBM CSLCs, for example using interferon- $\beta$  (IFN- $\beta$ ), might be particularly promising approaches to target the tumor stem cell compartment and suppress its tumorigenic potential.

Inhibitor of apoptosis (IAP) proteins represent a family of proteins that are typically expressed at high levels in human cancers.<sup>3</sup> For example, in GBM a correlation has been reported between high expression levels of IAP proteins and poor prognosis or treatment resistance.<sup>4,5</sup> A

series of small-molecule inhibitors have been developed with the aim of antagonizing IAP proteins; these inhibitors include mimetics of the endogenous IAP antagonist Smac.<sup>3</sup> Smac is a mitochondrial intermembrane space protein that is released into the cytosol upon the induction of apoptosis and promotes caspase activation and cell death by binding to and neutralizing IAP proteins.<sup>3</sup> Smac mimetics are currently under evaluation in early clinical trials. Although IAP proteins were initially reported to have antiapoptotic functions, there is mounting evidence showing that they are also involved in the regulation of several nonapoptotic processes, including differentiation.<sup>6</sup> However, at present little is known about the nonapoptotic properties of IAP proteins.

A recent study revealed a novel nonapoptotic function of a Smac mimetic. Tchoghandjian et al.<sup>7</sup> report that Smac mimetic at nontoxic concentrations regulates the differentiation of GBM CSLCs. Treatment with Smac mimetic caused morphologic changes such as cell elongation and neurite outgrowth that are characteristic of differentiated cells. At the molecular level, differentiation of GBM CSLCs into the astrocytic lineage is

documented by increased mRNA and protein levels of the astrocytic marker glial fibrillary acidic protein (GFAP). In comparison, no changes in the expression levels of neuronal markers were observed upon treatment with Smac mimetic. This Smac mimetic-triggered differentiation of GBM CSLCs is accompanied by downregulation of several stemness markers including CD133, Nanog, and Sox2, in line with an inverse correlation between differentiation and stemness. Mechanistic studies aiming to elucidate the underlying signaling pathways revealed that Smac mimetic stimulates activation of the transcription factor nuclear factor-kappa B (NF- $\kappa$ B). Further studies showed that NF- $\kappa$ B is critically required for Smac mimetic-mediated differentiation of GBM CSLCs, since blockage of NF- $\kappa$ B by overexpression of dominant-negative I $\kappa$ B $\alpha$  super-repressor also abolished differentiation of GBM CSLCs as well as upregulation of GFAP expression upon treatment with Smac mimetic. From a clinical perspective, it is particularly relevant that Smac mimetic stimulates differentiation and changes in stemness markers in malignant GBM CSLCs, but not in non-malignant neural stem cells.

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Submitted: 07/21/2014; Revised: 07/23/2014; Accepted: 07/25/2014

<http://dx.doi.org/10.4161/23723548.2014.960769>

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Although the mechanisms underlying these differential effects in normal and neoplastic stem cells are not yet known, these findings point to a potential therapeutic window that could be exploited in future clinical applications. Importantly, treatment with Smac mimetic reduces the clonogenicity of GBM CSLCs *in vitro* and *in vivo*, resulting in suppression of the tumorigenic potential of GBM CSLCs in orthotopic and subcutaneous mouse models and increased survival of the mice. Thus, this study provides new insights into nonapoptotic signaling pathways that

are regulated by Smac mimetics. Additionally, the novel ability of Smac mimetic to promote differentiation of GBM CSLCs by targeting their stem cell properties has important implications for future clinical applications of Smac mimetics as cancer therapeutics beyond the induction of cancer cell death. Moreover, since CSLCs have been implicated in tumor formation and treatment resistance in a variety of cancers, these findings are likely to have a broader relevance for cancer therapy.

#### Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgments

The expert secretarial assistance of C Hugenberg is greatly appreciated.

#### Funding

This work has been partially supported by grants from the Deutsche Forschungsgemeinschaft and IUAP VII (to SF).

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