

## **Overview of the progress and prospects of SMAC mimetics in cancers: Is it a silver bullet?**

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### **Abstract**

Loss of apoptosis results in the survival and uncontrolled proliferation of cancer cells. Basic and clinical researchers have dissected myriads of central regulators of apoptosis. Second mitochondria-derived activator of caspases (SMAC)/ direct inhibitor of apoptosis protein (IAP)-binding protein with low pI (DIABLO) has attracted phenomenal attention because of its amazing ability to trigger apoptotic death. Accordingly, different teams of interdisciplinary researchers are working on the design and development of SMAC mimetics which can significantly inhibit primary and secondary tumor growth.

**Key words:** SMAC mimetics, cancer treatment, apoptosis, metastasis, tumor growth inhibition

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## Introduction

Despite the constantly developing innovations in cancer treatment, the phenomenon of metastasis remains the main problem that takes away the chance for our patients to be cured and causes death. The only way to improve treatment success and clinical outcomes in cancer disease is to elucidate the molecular mechanism of metastases.

As a hallmark of cancer, tumor metastasis involves the migration and invasion of cancer cells from the primary tumor site, intravasation and dissemination, survival during circulation, extravasation into distant sites, adaptation in the distant tissue, conditional dormancy and then reactivation, and colonization (1).

Although a lower percentage of tumor cells enter into circulation and endure mechanical stress to survive, reaching target sites and developing into metastases, treatment of drug-resistant metastatic cancers is therapeutically challenging (2). Mutation in genes, tumor microenvironment and epigenetic changes, immune system and host cell behavior play an important role in metastasis biology (2-4).

The conversion of proto-oncogenes to an oncogene activates proliferative signals. This conversion is an important underlying cause of cancer initiation and progression, as well as the formation of metastases. It can occur in a variety of ways, such as the transition of these protooncogenes to the "on" state, and the development of fusion proteins by chromosomal translocation (5).

Tumor suppressor genes encode proteins that normally restrict cell division, inducing apoptosis, programmed cell death. The most important key points in the cell cycle are these suppressor genes (e.g., Rb). Any mutation in these genes is one of the main reasons for the onset of cancer and its spread by forming metastases (6).

Proteolytic enzymes such as Matrix Metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases. By breakdown of basement membranes and degradation of the Extracellular Matrix (ECM), they facilitate cancer-cell invasion into the surrounding tissue (7). In this sense, loss of cell adhesion also plays an important role. It has been shown that the loss of  $\beta$ -catenin and E-cadherin, the essential elements of adhesion, causes tumor invasion and initiates EMT (epithelial mesenchymal transition) (8).

Molecular and cellular factors within tumor microenvironments contribute considerably to the progression of metastasis. Importantly, these tumor microenvironments contain a number of cell types that competently drive tumor progression, angiogenic vascular cells, cancer stem cells, immune cells, extracellular vesicles and cancer associated fibroblasts (9). Cancer stem cells retain the characteristically unique ability to renew themselves and contribute critically during tumor progression (10).

Importantly, aberrant overproduction of proangiogenic factors by tumor cells results in tumor angiogenesis that facilitates tumor cell intravasation and dissemination. Tumor angiogenesis facilitates the hematogenous spreading of metastatic tumors.

Therefore, vascular endothelial growth factor (VEGF) and its receptor are among the targeted molecules in cancer therapy (11, 12).

One of the important factors playing a role in cancer biology and metastasis pathogenesis is the miRNA. miRNAs are involved in the regulation of initiation and progression of cancer metastasis (13).

The pathogenesis of metastasis, which is mainly responsible for the frightening consequences of cancer, is a multifactorial area that requires serious investigation. Only if the pathogenesis of metastasis is completely resolved will clinicians be able to achieve success in oncological treatments.

The development of SMAC-based peptidomimetics served as an important milestone in the journey of identification of chemicals that have extraordinary pharmacological properties, improved inhibitors of apoptosis proteins (IAP)-binding affinities, and the ability to induce apoptosis in drug-resistant cancer cells. Therefore, an important and crucial step in the design of these peptidomimetics is to improve and enhance the rigidity of the central scaffold.

Interdisciplinary researchers worked jointly and tested a compound which had a rigid bicyclic scaffold and N-methylalanine at P1 position. Interestingly, the results were valuable and this compound demonstrated a strong interaction with baculovirus IAP Repeat (BIR) domains of IAP, inducing apoptosis in cancer cells as a single agent (14). Contemporary studies paved the way for the evaluation of the therapeutic potential of different monovalent IAP antagonists that proficiently sensitize cancerous cells to a broader range of death-activating stimuli (15, 16).

Another category of SMAC mimetics are dimeric/bivalent compounds. These compounds are formed by a chemical linkage of two SMAC mimetics. It is relevant to mention that Lipinski's rules are not predictive of the efficacy value of bivalent IAP antagonists. Chemical and molecular analyses of the therapeutic value of bivalent SMAC mimetic compounds were encouraging and sparked an interest in gathering comprehensive and fool-proof evidence about the potential role of bivalent IAP antagonists as effective therapeutics agents. Consequently, these cutting-edge research findings opened new avenues for the pursuit of design and critical evaluation of a powerful class of IAP antagonists. In contrast to monovalent compounds, bivalent antagonists displayed higher binding affinities for IAP constructs. More importantly, these bivalent antagonists promoted the dimerization of BIR2–BIR3 constructs of c-IAP1 and BIR3 constructs of X-linked inhibitor of apoptosis (XIAP).

### **Use of SMAC mimetics for tumor inhibition**

Recurrent resistance to cisplatin therapy in ovarian cancer has emphasized the importance of combination treatment. In new research, a novel small molecule conjugate SW IV-134, which can link via peptide linkage with sigma-2 ligand SW43 of the SMAC mimetic, has been introduced. Interestingly, clinical data show sigma-2 receptor is abnormally unregulated in ovarian cancer, and sigma-2 ligands have shown promising

results in improving drug selectivity. A comparative study on the individual and combined treatments of cisplatin and SW IV-134 showed that the co-treatments were superior in inducing apoptosis. More importantly, the combinatorial therapy of cisplatin and SW IV-134 in patient-derived xenograft (PDX) models not only increased the survival of tumor-free mice to 60%, but also decreased the recurrence of tumors. The improved efficacy of cisplatin by the addition of SW IV-134 was consistent in in-vitro ovarian cancer cell lines, immunocompetent (Syngeneic mouse models) and immunocompromised hosts (PDX models). Referring to an independent study of the same lab, the author showed that the possible mechanism behind the induction of apoptosis was the up-regulation of  $\text{NF}\kappa\beta$ ,  $\text{TNF-}\alpha$ , caspases and inhibition of anti-apoptotic genes cIAP-1, cIAP-2 (17).

The recurrence of tumors in epithelial ovarian cancer patients is mostly due to the insensitivity towards standard platinum-based chemotherapy. The combined treatment with birinapant (a SMAC mimetic compound) and carboplatin was found to be effective in various platinum-resistant EOC cell lines, in tumor samples and in PDX models. To simulate in-vivo responses, 3D organoid bioassay was successfully employed to study the combined effect of birinapant and carboplatin, which demonstrated improved anticancer efficacy that suggests the applicability of this model in pre-clinical studies (18).

In another study, Lalaoui and colleagues have shown potential apoptotic effects of the SMAC-mimetic compound A and birinapant in triple-negative breast cancer cells (TNBCs) and in PDX models. Importantly, birinapant was more potent in TNBCs compared to estrogen receptor-positive ( $\text{ER}^+$ ) breast cancer, and genes related to SMAC-mimetic mediated apoptosis were found to be preferentially overexpressed in TNBCs compared to  $\text{ER}^+$  breast cancer. The formation of cell death complex in response to SMAC-mimetics in TNBCs (but not  $\text{ER}^+$ ) refers to the individualization of these cancer therapies based on patients with potent death receptor signalling (19).

Furthermore, birinapant induced antiproliferative and pro-apoptotic effects by the activation of cellular inhibitor of apoptosis 1 (cIAP1)/tumor necrosis factor receptor-associated factor 3 (TRAF3) axis in the liver cancer cell lines (Huh7, H22 and HepG2) and in nude mice with no effect on the hepatocyte line (20).

A study in the head and neck squamous cell carcinoma (HNSCC) illustrated the over-expression of inhibitor of apoptosis proteins (cIAP1) in human papillomavirus-HPV negative compared with HPV positive HNSCC tumours. This suggests cIAP1 as a potential prognostic and therapeutic target in a subset of HNSCC tumours. SMAC-mimetic LCL161 up-regulated the radiosensitivity of HPV negative HNSCC tumour xenografts by regulating cIAP1 (21).

Lower expression of Caspase 8 has been linked with resistance against apoptosis-inducing therapies. The addition of SMAC-mimetic to carboplatin and paclitaxel therapy increased the survival by decreasing the tumour growth to >50% in Caspase 8-deficient ovarian xenografts compared to wild-type (22).

Contrary to the other research, SMAC mimetic LCL161 has been shown to potently enhance the aggressiveness of lung cancer cells by the activation of NF- $\kappa$ B pathway. This suggests the need for individualization of SMAC mimetic therapies based on further investigations (23).

### **Use of SMAC mimetics for metastasis inhibition**

SM83, a SMAC mimetic, is a pan-IAP inhibitor, and studies provide proof-of-concept about its efficacy against primary and secondary tumors. The stimulation of epidermal growth factor receptor (EGFR) with epidermal growth factor (EGF) and transforming growth factor  $\alpha$  (TGF $\alpha$ ) increased the expression of SNAI2 (snail family transcriptional repressor 2). However, pre-treatment with cetuximab (EGFR-specific inhibitor) led to a notable reduction in EGFR and extracellular signal-regulated kinase 1/2 (ERK1/2) activation, and consequently limited EGF-mediated accumulation of SNAI2. cIAP1 depletion potently suppressed ERK1/2 phosphorylation and prevented the accumulation of SNAI2 upon treatment with EGF or TGF $\alpha$ . Intraperitoneal or intravenous injections of SM83 delayed the growth of primary tumors and also significantly reduced both the number and size of lung metastases in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice subcutaneously engrafted with MDA-MB-231 cells (24).

cIAP1/2 polyubiquitinate RIPK1 promoted the NF $\kappa$ B-mediated induction of target gene networks that promoted cellular proliferation, migration and invasion after treatment with TNF $\alpha$ . SMAC and its mimetics have been reported to effectively promote auto-ubiquitylation and degradation of cIAP1/2, which leads to the deubiquitylation of RIPK1 and consequently facilitates the formation of “ripiptosome” complexes. SMAC mimetics (LCL161, GDC-0152, SM-164) have considerable potential. Tumors in GDC-0152-treated mice were significantly smaller in size and less metabolically active compared to the untreated tumors. Administration of highest doses of GDC-0152 caused a reduction in their body weight, but they gradually recovered and regained similar weights (25). LCL161 and GDC-0152 potentially kill cells via blockade of XIAP-induced inhibition of caspases. Importantly, the underlying mechanism of lethality involved the activation of cIAP1/2 degradation, enhanced RIPK1 deubiquitylation, which redirected TNF $\alpha$ -induced TNFR1 cascade towards necroptotic or apoptotic pathways. A detailed investigation of TNF $\alpha$  levels revealed mechanistic insights in xenografted animal models. Blood and tumor tissues were harvested from mice that developed osteosarcomas. The findings clearly demonstrated that the spontaneous osteosarcomas were rich in TNF $\alpha$  similar to the subcutaneously implanted tumors. Importantly, osteosarcoma targeting ability of these SMAC mimetics is dependent on the production of TNF $\alpha$  by myeloid cells within the tumors. Fundamentally, SMAC mimetics proficiently killed disaggregated cells from freshly-resected implanted tumors (consisting of infiltrating non-cancerous cells and osteosarcoma cells). The scientific development of intratibial and intrafemoral osteosarcoma implantation models has opened new possibilities for deeper analysis of underlying mechanisms. However, intraosseous tumors have not been found to be well

tolerated by mice. Intramuscular implantation models are also being investigated for assessment of the effects of SMAC mimetics against osteosarcoma xenografts. Intramuscularly implanted osteosarcoma cells, either into the gastrocnemius muscle or hind paw, have been demonstrated to be highly tumorigenic in nature. To minimize the effects of tumorigenesis on leg functions, luciferase-expressing KRIB tumor cells were injected into the cranial tibial muscles in animal models. These strategies were well tolerated and yielded reproducible growth of primary tumors in animal models. LCL161 and doxorubicin led to a significant delay in the development of metastases. Treatment of mice either with doxorubicin alone or co-treatment with doxorubicin and LCL161 failed to develop pulmonary metastasis and significantly durable regression of the primary tumors (25).

### **Conclusion remarks**

SMAC mimetics have started to gain remarkable appreciation because of their encouraging results in cell-culture studies and xenografted animal model-based research works. Future research must converge on the identification of the most effective drug combinations for successful cancer treatment.

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# **Pregled razvoja i mogućnosti upotrebe SMAC mimetika: Da li se radi o magičnom oružju u lečenju karcinoma?**

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## **Kratak sadržaj**

Izostanak apoptoze dovodi do preživljavanja i nekontrolisanog umnožavanja ćelija karcinoma.

Istraživači koji se bave opštim i kliničkim ispitivanjima proučavali su mnoštvo centralnih regulatora apoptoze. SMAC/DIABLO (sekundarni mitohondrijski proizveden aktivator kaspaze) privukao je izuzetnu pažnju zahvaljujući izvanrednoj sposobnosti da izazove apoptotičku smrt. Stoga različiti timovi interdisciplinarnih istraživača rade na razvoju i usavršavanju SMAC mimetika koji bi mogli značajno da inhibiraju rast primarnih i sekundarnih tumora.

**Ključne reči:** SMAC mimetici, lečenje karcinoma, apoptoza, metastaza,  
inhibicija rasta tumora

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