Niclosamide: An Established Antihelminthic Drug as a Potential Therapy Against S100A4-Mediated Metastatic Colon Tumors

David M. Helfman

Correspondence to: David M. Helfman, PhD, Department of Biological Sciences and Graduate School of Nanoscience and Technology (WCU), Korean Advanced Institute of Science and Technology, Daejon 305-701, Korea (e-mail: dhelfman@kaist.ac.kr).

Progression of cancers because of the ability of primary tumor cells to grow outside their local environment and spread to distant sites (metastasize) is a major problem and a major cause of death from cancers. Patients with non-metastatic local stage I tumor have a 5-year survival rate of 90%, which drops to approximately 10% in patients with distant metastasis at the time of diagnosis. Therefore, it is important to identify genes that mediate metastasis and develop effective therapies against these potential targets.

S100A4 is a small calcium-binding protein of 101 amino acids in length that shows elevated expression in various cancers, including colon, breast, gastric, lung, colon, and liver, and is associated with metastasis. High levels of S100A4 expression are associated with poor prognosis, presumably because of its effect on metastasis formation. Studies in experimental mouse models have demonstrated that overexpression of S100A4 is capable of inducing metastasis and highly invasive primary tumors. S100A4 itself is not tumorigenic because transgenic mice expressing high levels of S100A4 do not develop tumors per se. However, when transgenic mice expressing high levels of S100A4 are mated with mice expressing mouse mammary tumor virus (MMTV) promoter-driven NEU (rodent ortholog of HER2) and exhibiting spontaneous tumor formation, the resulting female progeny expressing both S100A4 and NEU shows increased invasiveness of primary tumors and the appearance of metastasis in the lungs compared with littermates expressing only NEU. Furthermore, S100A4-null mice injected with highly metastatic mouse carcinoma cells show no metastasis. Thus, S100A4 appears to play a critical function in metastasis. Although the molecular mechanism underlying the prometastatic function of S100A4 is not fully understood, S100A4 has been shown to affect a number of cellular features associated with metastasis including epithelial–mesenchymal transition, enhanced migration and invasion, cell adhesion, angiogenesis, and survival. Given the potential role of S100A4 in mediating metastasis of human cancers, targeted therapies against the expression of S100A4 might be useful in treating tumors that show high S100A4 expression.

The canonical WNT/β-catenin (CTNNB1) signaling pathway is activated in about 80%–90% of sporadic colon tumors, and S100A4 is a transcriptional target of WNT signaling. In this issue of the Journal, Sack et al. (8) report that the drug niclosamide, which is widely used to treat helminthic infection, can inhibit S100A4 expression resulting in inhibition of cell migration and metastatic progression of colon cancer. The authors (8) carried out a high-throughput screen to identify pharmacologically active compounds that could inhibit transcription of S100A4 using a human colon cancer cell line, HCT116, expressing S100A4 promoter-driven luciferase reporter gene construct. Among the 1280 compounds tested, niclosamide was the strongest potential candidate for inhibition of the reporter gene expression. The authors further analyzed the effects of niclosamide on endogenous S100A4 expression in HCT116 cells and found that S100A4 mRNA and protein levels were reduced in a concentration-dependent manner.

A major function attributed to S100A4 in mediating metastasis is enhanced cell migration and invasion. Sack et al. (8) analyzed the effects of niclosamide on S100A4-induced cell migration of HCT116 cells. Assessment of cell migration using Boyden chamber and wound healing assays demonstrated that the treatment of parental HCT116 cells with niclosamide resulted in statistically significant inhibition of cell migration. Furthermore, because S100A4 is known to induce cell invasion, Sack et al. (8) performed a reconstituted basement membrane (Matrigel)-covered Boyden chamber assay to determine whether niclosamide affected the invasion of HCT116 cells through the basement membrane. Niclosamide-treated cells showed a decreased number of invading cells. Similar results were obtained using other human colon cancer cell lines that exhibit increased levels of S100A4 (SW620, LS174T, SW480, and DLD-1). The effects of niclosamide on inhibition of cell migration and invasion appear to be specific to S100A4 expression because migration of HCT116 cells expressing S100A4 under the control of the CMV-promoter were not affected by niclosamide, presumably because S100A4 levels were not decreased by niclosamide treatment.

Because S100A4 is a downstream target of WNT signaling, Sack et al. (8) investigated whether niclosamide treatment inhibited transcription of the WNT signaling pathway. The authors used a lymphoid enhancer–binding factor 1/transcription factor activity reporter assay to analyze the WNT/CTNNB1-dependent transcription of S100A4. Treatment of HCT116 cells with niclosamide showed reduced WNT signaling, which is in agreement with a recent study in osteosarcoma cells that also demonstrated inhibition of WNT signaling by niclosamide. Although the authors (8) demonstrate that niclosamide inhibits cell migration and invasion by decreased expression of S100A4, it is important to note that this study also shows that niclosamide inhibits cell proliferation and anchorage-independent colony formation independent of the level of endogenous or ectopic S100A4 expression, suggesting that genes other than S100A4 may mediate the effects of niclosamide. Indeed, a recent study showed that the antiproliferative effect of niclosamide on acute myelogenous leukemia stem cells was mediated by inhibition of nuclear factor of...
kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) signaling resulting in generation of reactive oxygen species and leading to apoptosis (10). Thus, niclosamide might be an effective target against both S100A4-dependent and S100A4-independent functions associated with cancer development via its effects on WNT or NFKB1 signaling pathways.

Finally, Sack et al. (8) investigated the effects of niclosamide on metastasis formation in a mouse xenograft model and used HCT116 cells to induce tumor formation. Mice harboring primary tumors in the spleen readily exhibited liver metastasis, which was not observed in niclosamide-treated mice. In addition, niclosamide treatment showed reduced expression of S100A4 mRNA and protein in the primary tumor. The long-term effects of niclosamide demonstrated reduced liver metastasis formation in mice and increased overall survival. Recently, another study (11) has also independently demonstrated that niclosamide inhibits activation of the WNT signaling pathway and elicits antitumor responses in cells derived from human colon tumors.

In summary, Sack et al. (8) demonstrates that niclosamide inhibits metastasis formation in a mouse xenograft model of colon cancer via regulation of WNT-dependent S100A4 expression and shows potential for clinical benefits in humans. Niclosamide, approved by the US Food and Drug Administration, is administered orally to treat patients with helminth infection. It still needs to be determined whether the antimetastatic activity of niclosamide can be achieved by oral or intravenous administration. Finally, given the range of tumors that overexpress S100A4 and exhibit alterations in WNT signaling, it is possible that niclosamide might be useful in treating a variety of human cancers, in addition to colorectal cancers.

References
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Affiliation of author: Department of Biological Sciences and Graduate School of Nanoscience and Technology, Korean Advanced Institute of Science and Technology, Daejon, Korea.