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Mini-review

Multi-targeted therapy of cancer by niclosamide: A new application for an old drug

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ABSTRACT

The rapid development of new anticancer drugs that are safe and effective is a common goal shared by basic scientists, clinicians and patients. The current review discusses one such agent, namely niclosamide, which has been used in the clinic for the treatment of intestinal parasite infections. Recent studies repeatedly identified niclosamide as a potential anticancer agent by various high-throughput screening campaigns. Niclosamide not only inhibits the Wnt/β-catenin, mTORC1, STAT3, NF-κB and Notch signaling pathways, but also targets mitochondria in cancer cells to induce cell cycle arrest, growth inhibition and apoptosis. A number of studies have established the anticancer activities of niclosamide in both *in vitro* and *in vivo* models. Moreover, the inhibitory effects of niclosamide on cancer stem cells provide further evidence for its consideration as a promising drug for cancer therapy. This article reviews various aspects of niclosamide as they relate to its efficacy against cancer and associated molecular mechanisms.

Introduction

Niclosamide (trade name Niclocide), a teniacide in the anthelmintic family which is especially effective against cestodes, has been approved for use in humans for nearly 50 years (Fig. 1) [1,2]. Niclosamide inhibits oxidative phosphorylation and stimulates adenosine triphosphatase activity in the mitochondria of cestodes (eg. tapeworm), killing the scolex and proximal segments of the tapeworm both *in vitro* and *in vivo* [2]. Niclosamide is well tolerated in humans. The treatment of *Taenia saginata* (beef tapeworm), *Diphyllobothrium latum* (fish tapeworm) and *Dipylidium caninum* (dog tapeworm) in adult is 2 g as a single oral dose. For the treatment of *Hymenolepis nana* (dwarf tapeworm), the same oral dose is used for 7 days [2].

Drug development, from the initial lead discovery to the final medication, is an expensive, lengthy and incremental process [3]. Finding new uses for old or failed drugs is much faster and more economical than inventing a new drug from scratch, as existing drugs have known pharmacokinetics and safety profiles and have often been approved for human use, therefore any newly identified use(s) can be rapidly evaluated in clinical trials [4]. In the last 5 years niclosamide has been identified as a potential anticancer agent by various high-throughput screening campaigns. This article reviews the current studies regarding various aspects of niclosamide as they relate to its potential new use in cancer therapy.

Niclosamide – a multiple pathway inhibitor for anti-cancer efficacy

Recently, several studies reported the inhibitory effects of niclosamide on multiple intracellular signaling pathways. The signaling molecules in these pathways are either over-expressed, constitutively active or mutated in many cancer cells, and thus render niclosamide as a potential anticancer agent. The effects of niclosamide on these pathways are described below.

The Wnt/β-catenin pathway

The Wnt/β-catenin signaling pathway regulates cancer progression, including tumor initiation, tumor growth, cell senescence, cell death, differentiation and metastasis [5–7]. In the absence of Wnt,

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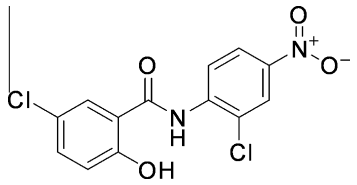

Fig. 1. The chemical structure of niclosamide.

β-catenin is sequestered in a complex that consists of the adenomatous polyposis coli (APC) tumor suppressor, axin, glycogen synthase kinase-3β (GSK3β), and casein kinase 1 (CK1). This complex formation induces the phosphorylation of β-catenin by CK1 and GSK3β, which results in the ubiquitination and subsequent degradation of β-catenin by the 26S proteasome. Conversely, when Wnt proteins form a ternary complex with the cell surface receptors, low-density lipoprotein receptor-related protein5/6 (LRP5/6) and Frizzled (Fzd), signaling from Wnt receptors proceeds through the proteins dishevelled (Dvl) and axin, leading to the inhibition of GSK3β and the stabilization of cytosolic β-catenin. The β-catenin then translocates into the nucleus where it interacts with T-cell factor/lymphoid enhancing factor (TCF/LEF) to induce the expression of specific target genes [5–7] (Fig. 2A).

Chen et al. performed a high-throughput screening of a library containing approximately 1200 FDA-approved drugs and drug-like molecules with a primary imaged-based green fluorescent protein (GFP) fluorescence assay that used Fzd1 endocytosis as the readout in human osteosarcoma U2OS cells, and identified niclosamide as a small molecule inhibitor of Wnt/β-catenin signaling [8]. Niclosamide promoted Wnt receptor Fzd1 endocytosis, downregulated Dvl2 protein, and inhibited Wnt3A-stimulated β-catenin stabilization and TCF/LEF reporter activity in U2OS cells [8]. It also decreased the cytosolic expression of endogenous Dvl2 and β-catenin in human colorectal cancer cell lines and in human colorectal cancer cells isolated by surgical resection of metastatic disease, regardless of mutations in *APC* [9]. Moreover, we recently demonstrated that niclosamide was able to inhibit Wnt/β-catenin signaling by promoting Wnt co-receptor LRP6 degradation, but had no effect on Dvl2 expression in prostate and breast cancer cells [10,11], suggesting that the mechanism underlying niclosamide-mediated inhibition of Wnt/β-catenin signaling could be cell type-dependent (Fig. 2A). In addition, the inhibitory effects of niclosamide on Wnt/β-catenin signaling were also demonstrated in primary human glioblastoma cells [12].

More than 90% of colorectal cancers bear mutations that result in the activation of the Wnt/β-catenin pathway [13]. S100 calcium binding protein A4 (S100A4) is a target gene of the Wnt/β-catenin pathway in colorectal cancers [14]. Sack et al. performed a high-throughput screening of the LOPAC chemical library containing 1280 compounds with a cell-based luminescence assay that used S100A4 promoter-driven luciferase activity as the readout in human colorectal cancer HCT116 cells, and identified niclosamide as an inhibitor of S100A4 through reduction of S100A4 mRNA and protein expression in colorectal cancer cells [15]. It was proposed that niclosamide inhibits β-catenin/TCF complex formation and thereby interrupts target gene transcription, an alternative model of Wnt/β-catenin inhibition by niclosamide in colorectal cancer cells [15] (Fig. 2A).

The mTORC1 pathway

The mammalian target of rapamycin complex 1 (mTORC1) is a heterotrimeric protein kinase that consists of the mTOR catalytic subunit and two associated proteins, raptor (regulatory associated protein of mTOR) and mLST8 (mammalian lethal with sec-13). The mTORC1 activity is regulated by upstream signals from growth factors, amino acids, stresses and energy state, and its activation induces the phosphorylation of p70S6 kinase (p70S6K) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), leading to the enhanced translation of a subset of mRNAs that are critical for cell growth and metabolism [16,17] (Fig. 2B). Activation of either the serine/threonine protein kinase Akt (also known as protein kinase B or PKB) or the extracellular signal-regulated kinase (ERK) pathway, or inhibition of the adenosine monophosphate-activated protein kinase (AMPK) pathway, leads to activated mTORC1signaling. As a downstream effector of Akt, mTORC1 has been described as the most essential effector in driving cell proliferation and susceptibility to oncogenic transformation. This leads to the targeting of mTORC1 as a therapeutic strategy in many types of cancer [16,17].

Balgi et al. performed a high-throughput screening of a collection of >3500 chemicals with a primary imaged-based enhanced GFP (EGFP) fluorescence assay that used EGFP-LC3 punctate staining as the readout in human breast cancer MCF-7 cells, and identified niclosamide as an inhibitor of mTORC1 signaling in cells maintained in nutrient-rich conditions [18]. Niclosamide did not inhibit mTORC2, which also contains mTOR as a catalytic subunit, suggesting that niclosamide does not inhibit mTOR catalytic activity but rather inhibits signaling to mTORC1 [18]. Tuberous sclerosis complex (TSC2), a negative regulator of mTORC1, was not required for the inhibition of mTORC1 signaling by niclosamide, as niclosamide was able to suppress mTORC1 signaling in TSC2-deficient cells where mTORC1 activity was elevated [18]. Further studies demonstrated that niclosamide does not impair PI3K/Akt signaling, nor does it interfere with mTORC1 assembly and mTORC1 kinase activity [19]. It had been proposed that increased cytosolic acidification is responsible for the mTORC1 inhibition, and that the structural features of niclosamide required for protonophoric activity are essential for the mTORC1 inhibition [19]. Lysosome is the degrading machine for autophagy, but has also been linked to mTORC1 activation through the Rag/RRAG GTPase pathway [20]. More recently, Li et al. proposed that mTORC1 inhibition by niclosamide is caused by lysosomal dysfunction [21]. Niclosamide inhibits the lysosomal degradative function likely by altering lysosomal permeability and the pH gradient [21], which is consistent with the finding that niclosamide can cause dispersion of protons from the lysosomes to the cytosol, leading to cytosolic acidification [19] (Fig. 2B).

The STAT3 pathway

Signal transducers and activators of transcription 3 (STAT3), a member of a family of six different transcription factors, is one of the down-stream signaling proteins for cytokine and growth factor receptors [22]. Engagement of cell-surface cytokine or growth factor receptors activates the janus kinase (JAK) family of protein kinases, which in turn phosphorylates STAT3 at tyrosine residue 705, leading to the dimerization of two STAT3 monomers through the SH2 domains of the proteins [23,24]. The activated STAT3 dimers then translocate into the nucleus and activate the transcription of a panel of genes that control cell proliferation, apoptosis, angiogenesis and other cell functions [25,26]. This JAK2/STAT3 signaling pathway is one of the three major modules that play an essential role in transmitting external signals from the surface membrane to target genes in the nucleus, controlling processes such as growth, differentiation, senescence and apoptosis [27,28]. Importantly, STAT3 is the major intrinsic pathway for cancer inflammation owing to its frequent activation in malignant cells and key role in regulating many genes crucial for cancer inflammation in the tumor microenvironment [22].

Ren et al. demonstrated that niclosamide is a potent STAT3 inhibitor that suppresses STAT3 transcriptional activity by