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Engineering of caveolae-specific self-micellizing anticancer lipid nanoparticles to enhance the chemotherapeutic efficacy of oxaliplatin in colorectal cancer cells

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ABSTRACT

Novel nanomaterials for the intracellular transport of therapeutic cargos have been actively sought to effectively breach cell-membrane barriers. In this study we developed novel self-micellizing anticancer lipid (SMAL)-based *pro-apoptotic* nanoparticles (NPs) that enhance the accumulation and chemotherapeutic efficacy of oxaliplatin (OL) in colorectal cancer cells (CRCs). We demonstrated that NPs with special affinity to *caveolae* could be designed and based on this specificity, NPs effectively differentiated between endothelial cells (tumor cells) and epithelial cells, without the need for a cell-specific targeting moiety. We demonstrated a remarkable uptake of OL-loaded SMAL NPs (SMAL-OL) in HCT116 and HT-29 cells via the caveolae-mediated endocytosis (CvME) pathway. The higher accumulation of SMAL-OL in the intracellular environment resulted in a significantly elevated anticancer effect compared to that of free OL. Cell cycle analysis proved G2/M phase arrest, along with substantial presence of cells in the sub-G1 phase. An immunoblot analysis indicated an upregulation of pro-apoptotic markers (Bax; caspase-3; caspase-9; and PARP1) and downregulation of Bcl-xl and the PI3K/AKT/mTOR complex, indicating a possible intrinsic apoptotic signaling pathway. Overall, the ability of SMAL NPs to confer preferential specificity towards the cell surface domain could offer an exciting means of targeted delivery without the need for receptor-ligand-type strategies.

Statement of Significance

In this work, we developed a novel self-micellizing anticancer lipid (SMAL)-based pro-apoptotic nanoparticles (NPs) that enhance the accumulation and chemotherapeutic efficacy of oxaliplatin (OL) in colorectal cancer cells. We demonstrated that NPs with special affinity to caveolae could be realized and based on this specificity, NPs effectively differentiated between endothelial cells (tumor cells) and epithelial cells, without the need for a cell-specific targeting moiety. In addition, oxaliplatin-loaded SMAL were efficiently endocytosed by the cancer cells and represent a significant breakthrough as an effective drug delivery system with promising potential in cancer therapy. We believe this work holds promising potential for the development of next generation of multifunctional nanocarriers for an exciting means of targeted delivery without the need for receptor-ligand-type strategies.

1. Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths, with approximately 50,000 deaths in the United States alone and around 650,000 deaths worldwide per year [1]. Gastrointestinal (GI) tract cancer is the second leading cause of death among male and female patients. Overall, the lifetime risk of developing CRC is about 1 in 20 (5.1%) [2]. Surgery is commonly employed as a curative treatment in various stages of early, as well as advanced, CRC. However, surgery can not eliminate all malignant cells, therefore warranting additional drug therapies such as chemotherapy [3].

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The Food and Drug Administration approved oxaliplatin (OL), a third-generation platinum compound, for the treatment of stage III, advanced, or metastatic CRC [4]. OL acts by binding to DNA or RNA, and forms intrastrand adducts (platinum-DNA adducts), which disrupt DNA replication and transcription processes. This results in cell apoptosis, and eventually cell death [5]. However, the use of OL is still associated with a range of side effects, including myelotoxicity, acute and chronic peripheral neuropathy, cardiotoxicity, and severe GI tract disorders [6]. Moreover, conventional chemotherapeutic drugs often suffer from poor solubility, a narrow therapeutic window, and poor systemic distribution patterns, which may result in treatment failure in cancers. In order to improve the therapeutic efficacy and reduce the toxicities of platinum drugs, various delivery systems (liposomes, polymeric nanoparticles, polymeric capsules and inorganic nanoparticles) have been studied, which can direct the anticancer drugs to solid tumors by virtue of the enhanced permeation and retention (EPR) effect [7,8]. Nevertheless, a lack of adequate cellular uptake, carrier-mediated toxicity and poor dispersion stability of nanocarriers has hampered the clinical translation of OL in CRC [9].

Nanoparticulate drug delivery systems (Nano-DDS) have emerged as promising strategy for the specific delivery of anticancer drugs [10,11]. After circulating in the bloodstream, nanoparticles (NPs) could passively accumulate in the tumor tissue [12]. In this context, any nanomaterial that can enhance the chemotherapeutic response of a drug moiety, while being non-toxic to normal cells, is highly desired (a pro-apoptotic agent) [13,14]. Bioactive lipids such as sphingolipids play an important role in signal transduction pathways, especially growth arrest, cell proliferation and pro-apoptotic effects in cancer cells [22]. They control various membrane-associated signaling pathways, which are actively involved in growth and apoptosis of cancer cells. Moreover, sphingolipids have been reported to induce apoptosis by generating reactive oxygen species (ROS), caspase expression and Bax translocation. Recently, it has been established that sphingolipid-based novel self-micellizing anticancer lipid (SMAL) could selectively induce cell death via the activation of apoptosis and autophagy in CRC cell lines [15,16]. Through understanding this clinical outcome, we sought to develop a SMAL-based Nano-DDS that serves the dual function of a drug delivery agent with antitumor activity.

Recently, great efforts have been made to identify effective and new pharmacological targets that can increase cancer-targeting efficiency [17]. Despite the great advancement in cancer biology, a generalized approach of targeting the therapeutic agent to malignant cells is a big challenge due to the variability in the expression of targets in different patients and at different stages of cancers [18]. Therefore, a highly generalized approach, which can differentiate between various cell types found within the tumor microenvironment, without the need for ligand/receptor-based active targeting, could be of great importance. In this context, lipid raft-based cellular uptake (through caveolae) is one of the processes involved in cell uptake of extracellular particles. Caveolin-1(CAV1) is a major structural protein in the caveolae (flask-shaped invaginations of the plasma membrane) and is involved in various cellular functions such as signal transduction, lipid metabolism, cell growth, survival and apoptosis [19,20]. The biological importance of caveolae stems from the difference in the caveolae density and expression levels of CAV1 in different cell types and tissues. For instance, reduced expression of CAV1 has been observed in ovarian, lung, head and neck carcinomas [21], while high expression of CAV1 was found to be associated with the progression of colon and prostate cancers. Importantly, low expression of CAV1 occurs in normal, healthy cells, making it an ideal pharmacological target [22,23].

Thus far, main aim of present study was to increase the therapeutic efficacy of OL in CRCs by loading it in a SMAL carrier. Based on the simple premise, it was hypothesized that OL-loaded SMAL NPs (SMAL-OL) could be specifically taken up by cancer cells without the need for cell-specific targeting ligands. This could be realized based on the specificity of the SMAL NPs towards the caveolae, which are overexpressed in CRC, while it can inherently discriminate the healthy cells due to the low expression of CAV1 (Fig. 1).

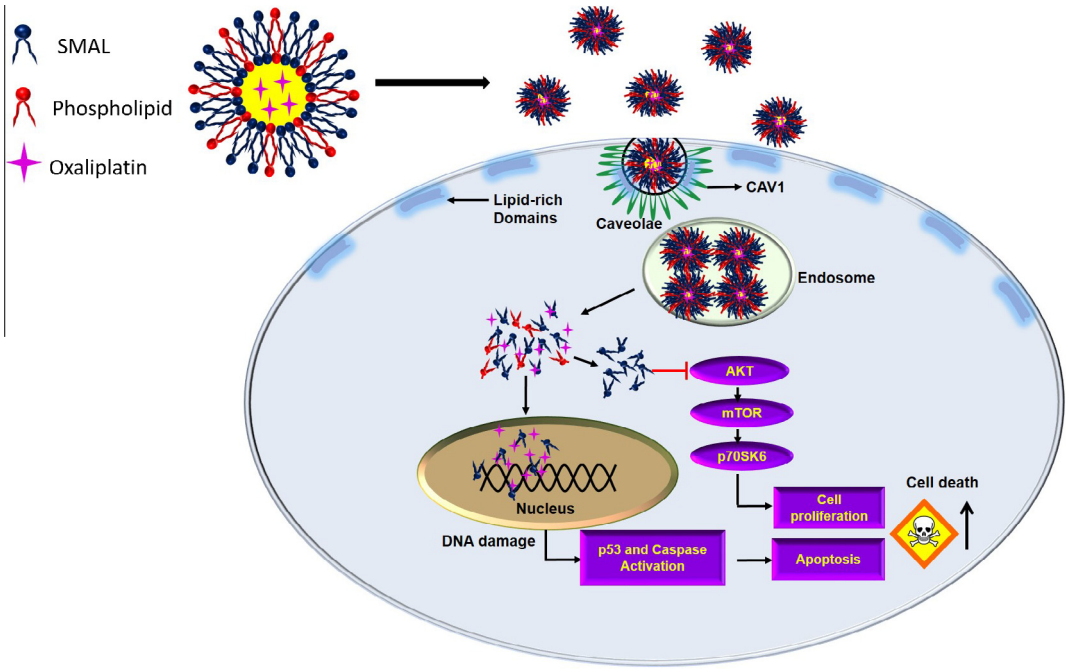

Fig. 1. Schematic illustration of preparation of oxaliplatin (OL)-loaded SMAL NP (SMAL-OL) and its effect on the cell signaling pathways in the intracellular environment.