Microbial amphiphiles: a class of promising new-generation anticancer agents

Goutam Dey[1], Rashmi Bharti[1], Ramkrishna Sen[2] and Mahitosh Mandal[1]

[1] School of Medical Science & Technology, Indian Institute of Technology Kharagpur, India
[2] Department of Biotechnology, Indian Institute of Technology Kharagpur, India

Developing new classes of anticancer molecules has always been a major scientific challenge owing to multidrug resistance of cancer cells to conventional chemotherapeutic agents. Microbial amphiphiles, particularly lipopeptides and glycolipids, have recently emerged as potential new-generation anticancer agents, owing to low toxicity, high efficacy and easy biodegradability. They exhibit anticancer activities by retarding cell cycle progression, inhibiting crucial signaling pathways such as Akt, extracellular signal-regulated kinase/c-Jun N-terminal kinase (ERK/JNK) and Janus kinase/signal transducer and activator of transcription (JAK/STAT), reducing angiogenesis, activating natural killer T (NKT) cells and inducing apoptosis through death receptors in cancer cells. It has been well established that the oncogenic signals of cancer cells are amplified by the overexpression of various membrane-bound receptors such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR) and insulin-like growth factor receptor (IGFR). Microbial amphiphiles, upon interaction with the cell membrane, are believed to suppress the activities of these cell surface receptors by fatty acid chain mediated membrane destabilization. This review analyzes the modes and mechanisms of action of these green molecules for application as potential anticancer agents.

Introduction

Cancer is supposedly one of the greatest concerns for human health owing to the unpredictable nature of its causes and manifestation. The chemotherapeutic treatment options in practice are limited in terms of safety and efficacy, because of high toxicity and activation of several resistant pathways in cancer cells after a few days of exposure. Although the world has witnessed huge investments and sustained efforts in cancer research to develop anticancer drugs in the past few decades, remarkable success for a well-targeted selective therapy devoid of toxicity has not yet been achieved. This has necessitated a constant R&D drive to discover new effective and safe anticancer molecules. Some natural compounds of plant origin are already known for their use in cancer therapy such as taxol, vinca alkaloids and etoposides [1]. However, producing therapeutic molecules from plant sources does not seem to be an attractive business proposition because of the following reasons: (i) plants have relatively low growth rates; (ii) genetic engineering of plants is not as easy as bacteria; (iii) plants require large amounts of land and water. Therefore, resorting to microbes with relatively higher growth rates as sources for the anticancer molecules appears to be a more rational approach because they can be genetically modified with ease and they do not require much land or water. There are many reports on the production of anticancer therapeutics by bacteria. For example, actinomycin D and doxorubicin are produced by the soil bacteria *Streptomyces* sp. [2,3].

Microbial amphiphiles are reported to possess tremendous commercial and therapeutic application potential [4]. The majority of the amphiphiles are extracellular secondary microbial metabolites that have been reported to have significant biological activities including antimicrobial, antifungal, antiviral and antimycoplasma

Corresponding authors: Mandal, Sen, R. (rksen@yahoo.com), (rksen@hijli.iitkgp.ernet.in), M. (mahitosh@smst.iitkgp.ernet.in)

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2014.09.006

[4]. These interesting molecules are also emerging as new-generation anticancer agents and drawing great attention from the global scientific community because of their high efficacy, biodegradability and low toxicity [4–6]. Some lipopeptides, for example, somocystinamide A, rakicidin, apratoxin and fellutamides, are highly cytotoxic to different cancer cell lines as compared with conventional chemotherapeutic drugs.

Deregulation and amplification of various signaling pathways such as phosphoinositide-3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK)/p38 c-Jun N-terminal kinase (JNK), cytokine-induced Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) and receptor tyrosin kinase (RTK) pathways generally lead to cancer formation and development of chemoresistance [7,8]. Inhibiting these pathways can be a molecular perspective for cancer therapy. Interestingly, many lipopeptides are also reported to interfere with these crucial signaling pathways [6,9,10].

Matrix metalloproteinases (MMPs) are a group of zinc- and calcium-dependent endopeptidase enzymes. Upregulation of MMP-2/9 has an essential role in cancer invasion and metastasis [11]. Secretion of this enzyme was inhibited by surfactin treatment in breast cancer cells suggesting its anti-invasive and antimetastatic properties [11]. So, microbial amphiphiles inhibiting these deregulated signaling pathways in cancer cells can be promising new molecules as anticancer agents. This article thus attempts to critically review the application potential of various microbial amphiphiles in cancer therapy, their mechanisms of action and bottlenecks in realizing their full potential as anticancer drugs and future directions.

Types of amphiphiles and their anticancer properties

Chemically, amphiphiles consist of hydrophobic saturated or unsaturated fatty acids and hydrophilic peptides or a polysaccharide. Amphiphiles are purified and characterized from culture media of various microorganisms. The commercial potential of these molecules has not yet been fully realized owing to poor yields and constraints regarding obtaining ultrapure product for therapeutic application [12]. However, the production yields of microbial a*mphiphiles* can be enhanced by media and process optimization [13,14]. Microbial amphiphiles can be produced using petroleum hydrocarbon, glucose, sucrose and glycerol as substrates [15,16]. Microbial amphiphiles include lipopeptides, phospholipids and glycolipids, as well as other types of molecules.

Structure–function relation of microbial amphiphiles

Microbial amphiphiles display significant surface and emulsifying activities. These molecules increase the surface area as well as reduce surface tension at the interface. They also partition at the interface between two different phases. In aqueous environments, amphiphiles form structured aggregates called micelles. The concentration at which micelles are formed is known as the critical micelle concentration (CMC), which is crucial for biological activity [17]. These molecules are represented by different types of molecules with diverse properties and biological functions. Microbial amphiphiles are believed to interact with the cell membrane and manifest various bioactive properties. They interact with many membrane macromolecules such as lipopolysaccharides [18], phosphatidylserine [19] and other membrane components [20]. Structural features including fatty acid chain length, amino acid composition, functional groups and type of sugar moiety greatly influence the biological activity of amphiphiles. We have summarized a number of amphiphiles and there structure–function relations in Table 1 [21–29].

Lipopeptides

Lipopeptides are complex molecules composed of lipid moieties connected to a peptide chain. Lipopeptides are reported to have significant biological activities such as antifungal, antibacterial, antiviral and antitumor activities [30,31]. Daptomycin and polymixin B are two microbial-derived lipopeptide antibiotics that are FDA-approved antimicrobial agents. Here, we discuss the anticancer properties and molecular mode of action of various lipopeptide molecules (Table 1). Among the reported lipopeptides, surfactin, iturin and fengycin are well known biosurfactants.

Surfactin

Among the reported lipopeptides, surfactin has been the most extensively explored. It is produced from various strains of *Bacillus subtilis* during fermentation [32] and composed of seven amino acids and a lipid part. Surfactin is synthesized through a unique nonribosomal protein synthesis catalyzed by a multi-enzymatic complex called surfactin synthetase [32]. The biosynthesis of surfactin is encoded by the *Srf* operon containing four open reading frames that encode different enzymes [32]. The four enzymatic subunits of surfactin synthetase are SrfA, SrfB, SrfC and SrfD [32,33]. Among them, SrfD starts the surfactin initiation reaction [33]. Surfactin is a lipopeptide consisting of 3-hydroxy-13-methyl-tetra decanoic acid amidated to the N-terminal amine of a heptapeptide moiety with the carboxyl terminal end of the peptide being further esterified to the hydroxyl group of the fatty acid. Structural characteristics revealed the presence of a heptapeptide with a LLDLLDL chiral sequence linked, via a lactone bond, to a hydroxy fatty acid. The antitumor activity of surfactin has been associated with the hydrophobic nature of the fatty acid component that interacts with the acyl chain of membrane-bound phospholipid [17]. Simultaneously, its peptide moiety strongly interacts with the polar heads of the membrane lipids in cancer cells. Surfactin, having a longer fatty acid chain, penetrates more efficiently into the cancer cell membrane [17].

A recent study has demonstrated the anti-invasive and antimetastatic actions of surfactin on MCF-7 breast cancer cells through inhibiting MMP-9 [11]. MMPs are the protease enzymes that promote tumor invasion and metastasis through degradation of matrix collagen. This lipopeptide also inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced nuclear factor (NF)-κB and activator protein (AP)-1 activation and nuclear translocation [11]. Further, it caused sustained inhibition of PI3K/Akt and MAPK signaling pathways in MCF-7 cells. It also caused reduction of unsaturation of fatty acid composition in the cell membrane that leads to cell apoptosis in Bcap-37 breast cancer cells [17]. Surfactin purified from *Bacillus natto* was first reported to have antitumor effects on Ehrlich ascites carcinoma cells [34] and this lipopeptide purified from the TK-1 strain has been shown to inhibit MCF-7 human breast cancer cells in a dose- and time-dependent manner [35]. Interestingly, it was found to be nontoxic in the normal cell lines BRL (buffalo–rat liver) and HEK-293 (human embryonic