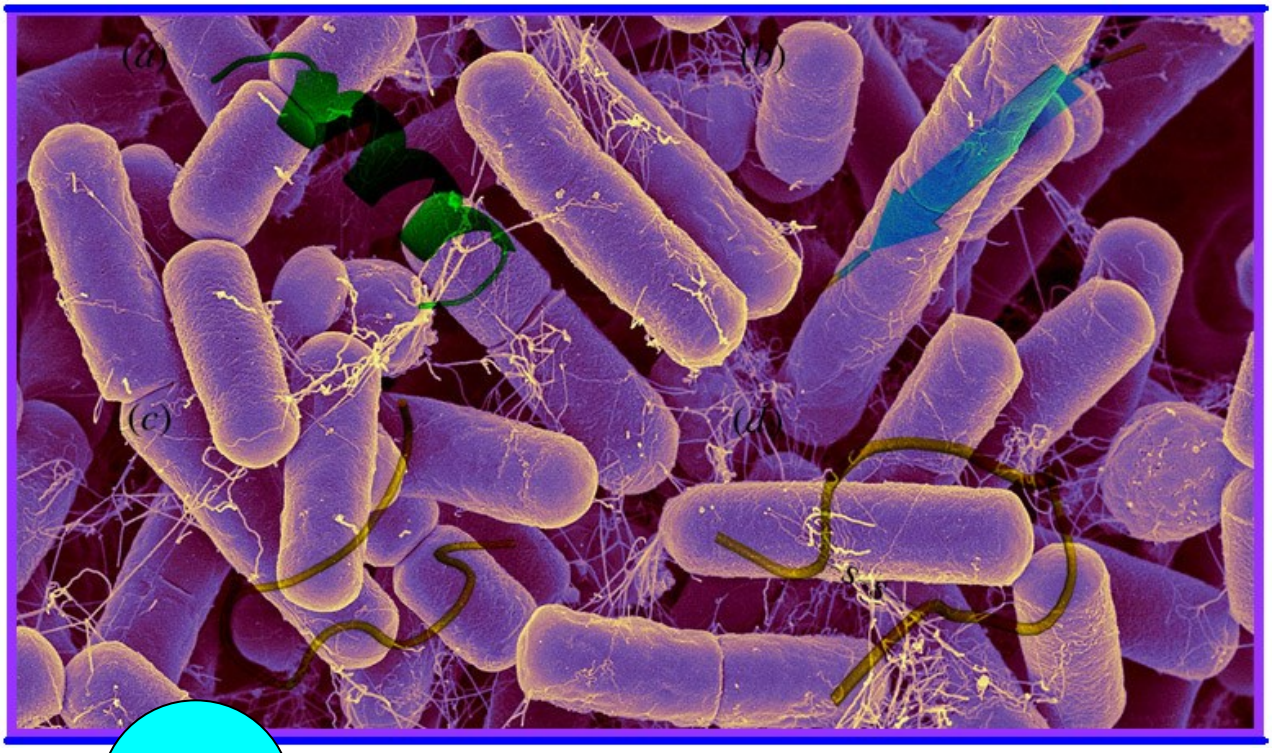

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Mini-review

Anti-cancer peptides from bacteria

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Abstract

Cancer is a leading cause of death in the world. The rapid development of medicine and pharmacology allows to create new and effective anti-cancer drugs. Among modern anti-cancer drugs are bacterial proteins. Until now has been shown anti-cancer activity among others azurin and exotoxin A from *Pseudomonas aeruginosa*, Pep27anal2 from *Streptococcus pneumoniae*, diphtheria toxin from *Corynebacterium diphtheriae*, and recently discovered Entap from *Enterococcus* sp. The study presents the current data regarding the properties, action and anti-cancer activity of listed peptides.

Introduction

Attempts to use bacteria or their products for the treatment of cancer dates back to turn of the XIX and XX century. William Coley (Coley, 1909) in the treatment of patients with unresectable tumors applied the treatment with bacterial culture supernatants of *Streptococcus pyogenes* and *Serratia marcescens*. This preparation called Coley's toxins was used in approximately 1200 patients with malignancy, often yielding regression of the tumor and in 30 patients, supposedly - a complete cure. Currently, it is assumed that the main factor responsible for therapeutic effect of Coley's toxins was induction of enhanced tumor necrosis factor-α (TNF-α) secretion in the body of the patient. The anti-tumor efficacy of TNF-α was confirmed in an animal model, observing the inhibition of growth and even complete regression of the tumor (Gratia and Linz, 1931; Carswell et al., 1975). Some studies have shown that the pathogenic microorganisms may proliferate inside hypoxic cancer lesions, simultaneously stimulating the immune system of the host during infection, leading to inhibition of cancer progression. An example would be a vaccine strain BCG (*Mycobacterium bovis* Calmette-Guerin), used in the treatment of superficial bladder cancer (Alexandroff et al., 1999) or attenuated *Salmonella typhimurium* (Pawelek et al., 1997), and anaerobic bacteria of the *Clostridium* genus (Dang et al., 2001) leading to enhanced tumor regression in mice. Currently, it is considered that the selected microbial infections can lead to activation of macrophages and lymphocytes, and result in the production of cytotoxic agents, especially TNF-α, having anti-cancer activity (Patyar et al., 2010). Currently, hope in the treatment of cancer relies, among other, on low molecular weight proteins, including those produced by microorganisms. In this paper, are presented basic anticancer peptides of bacterial origin. Selected characteristics of the below described peptides are presented in Tables I and II.

Azurin

Azurin (14 kDa, 128 amino acids) is a copper-containing single-domain protein with a rigid β-barrel structure, produced by *Pseudomonas aeruginosa*. Azurin contains a hydrophobic patch (van de Kamp et al., 1990). Azurin is a member of the cupredoxin family of redox proteins (Adman, 1991). The copper in azurin can be eliminated, creating apo-azurin (Nar et al., 1991). The apo-azurin has a very low redox activity (0.02 and 0.01% of the holoenzyme wt azurin) but demonstrates significant

Table I

Origin, molecular weights and structure of described anticancer peptides. For the visualization of the peptides structures were used modelling servers: I-TASSER http://zhanglab.ccmb.med.umich.edu/I-TASSER/ and SWISS-MODEL http://swissmodel.expasy.org/ (Kiefer et al., 2009)

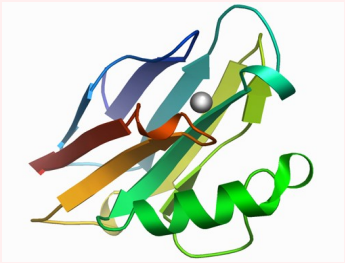
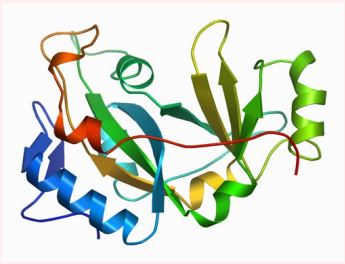
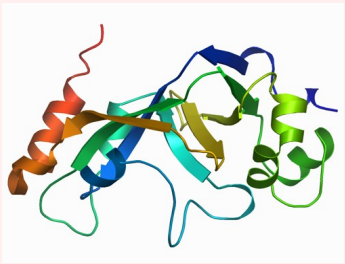
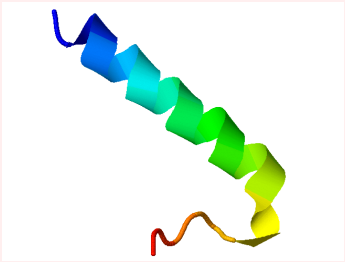
Peptide	Origin	Molecular weight	Structure
Azurin	*Pseudomonas aeruginosa strains*	14 kDa	 128 amino acids
Exotoxin A	*Pseudomonas aeruginosa strains*	66 kDa	 638 amino acids
Diphtheria toxin	*Corynebacterium diphtheria strains*	60 kDa	 538 amino acids
Pep27anal2	*Streptococcus pneumoniae strains*	3.3 kDa	 27 amino acids:
Entap	*Enterococus sp. strains*	6.2 kDa	58-62 amino acids (the sequence remains to be studied)

Table II

Target tumor cells and mode of cytotoxic action of described anticancer peptides

Peptide	Target tumor cells	Mode of cytotoxic action
Azurin	Breast cancer, melanoma, squamous carcinoma, reticulum cell sarcoma	Deregulation of proliferation and induction of caspase-dependent apoptosis
Exotoxin A	Pancreatic cancer, melanoma, head and neck squamous carcinoma, lung carcinoma, breast carcinoma, multiple myeloma	Inhibition of protein synthesis (ADP-ribosylation of cytoplasmic elongation factor 2)
Diphtheria toxin	T cell lymphoma, glioblastoma, malignant brain tumors, adrenocortical carcinoma	Inhibition of protein synthesis (ADP-ribosylation of cytoplasmic elongation factor 2)
Pep27anal2	Leukemia, gastric cancer, breast cancer	Cellular permeabilization, deregulation of proliferation and induction of caspase-independent apoptosis
Entap	Gastric adenocarcinoma, uterine cervix adenocarcinoma, mammary gland adenocarci-noma, prostate carcinoma, colorectal adenocarcinoma	Deregulation of proliferation and induction of autophagous apoptosis

cytotoxicity (Goto et al., 2003). Ability of azurin to destroy tumor cells appears to rely on the stability of the p53 protein which inhibits the development of the cancer. In nucleus azurin enhances the intracellular levels of p53 and Bax, what triggers the release of mitochondrial cytochrome c into the cytosol. This process activates the caspase cascade (including caspase-7 and caspase-9) initiating the apoptotic process (Punj et al., 2004). Apoptosis is an essential process in cellular development and in maintaining cellular homeostasis. Azurine much more effectively penetrates into the tumor cells than into the healthy. By this process is probably responsible domain Azu 50-70 (p28), consisting of 28 amino acids, and has molecular weight 2.8 kDa (Bernardes et al., 2013). p28 preferentially enter the human breast cancer cell lines MCF-7, ZR-75-1, and T47D through a caveolin-mediated pathway. p28 and similar peptides that reduce degradation of p53 may provide a unique series of cytostatic and cytotoxic chemotherapeutic agents (Yamada et al., 2009). Studies have shown direct antiangiogenic effect of p28 on endothelial cells. p28 preferentially penetrated human endothelial cells and inhibited VEGF- and bFGF-induced migration, capillary tube formation and neoangiogenesis (Mehta et al., 2011). This suggests that p28 is a unique agent possessing both antiangiogenic and cytostatic properties. Azurin binds to the NH_2-terminal domain and DNA-binding domain (DBD) of p53, but not the COOH-terminal region of p53 (Punj et al., 2004; Apiyo and Wittung-Stafshede, 2005). The azurin:p53 complex is stable (Taranta et al., 2008). Vasu et al. showed that azurine acts strongly cytotoxic, with respect to the breast cancer MCF-7 cell line, causing more than 50% increase in intensity of apoptosis. However, in the breast cancer MDA-MB-157, MDD2 and MDA-MB-231 cells, the increase in intensity of apoptosis was 15-18% (Vasu et al., 2007). It has been shown that in p53 wild-type MCF-7 breast cancer cells, treatment with azurin caused an increase in p53 levels in both nuclear and cytoplasmic fractions (Punj et al., 2004; Yamada et al., 2004; Yamada et al., 2009). Yamada et al. conducted a study in which over 22 days in immunocompromised mice and transplanted human melanoma UISO-Mel-2 cells was azurine administered. It was found that in mice treated with azurine there was a reduction in tumor size compared to mice not treated and there have been no fatal cases (Yamada et al., 2002a; Yamada et al., 2002b). In other studies, the azurin -treated oral squamous carcinoma cells showed decreased viability, morphological changes, DNA breakage, and increase in p53 and cyclin B1 protein levels. Increase of oral squamous carcinoma cells sensitivity to anticancer drugs was found in combination treatment of azurin with other anti-cancer agents (Choi et al., 2011). Azurin induces a p53-mediated apoptosis also in mammalian J774 macrophage-like cells from reticulum cell sarcoma. A high level of reactive oxygen species, generated during treatment of macrophages with azurin, correlates with its cytotoxicity (Yamada et al., 2002b). The studies showed that intravenous administra -tion of azurin p28 to mice showed its $t_{(1/2\ beta)}$ 0.23 h, clearance 1.7 l/kg/h, and volume of distribution at steady state 4.1 l/kg (Gorman et al., 2010). p28 does not exhibit preclinical immunogenicity or toxicity. The no observed adverse effect level was 120 mg/kg iv in female mice. In non-human primates no observed adver -se effect level of p28 was defined as the highest studied dose (120 mg/kg/dose; 1,440 mg/m^2/dose) (Jia et al., 2011). p28 has undergone phase I clinical trial (http://www.cdgti.com; IND 77754).

Exotoxin A

Exotoxin A (66 kDa, 638 amino acids) is the main toxin secreted by *Pseudomonas aeruginosa*. Its operation is identical with diphtheria toxin. It has activity of ADP-ribosyl transferase. It inactivates elongation factor-2 (EF -2) resulting in inhibition of protein synthesis. Exotoxin A production is dependent on the presence of iron. Exotoxin A contains four functional domains: The

receptor binding domain (Ia; aa 1–252), the translocation domain (II; aa 253–364), the domain Ib (aa 365–404), and the cytotoxic domain (aa 405–613) (Wolf and Elsässer-Beile, 2010). Exotoxin A is usually used as an immunotoxin, in combination with various ligands. Deimmunized *Pseudomonas* exotoxin cloned with human epidermal growth factor (EGF) and interleukin 4 had anti-cancer effect on orthotopic MIA PaCa-2 pancreatic cancer and selectively prevented metastasis (Oh et al., 2012). Exotoxin A-based immunotoxin, the 9.2.27PE, and the BH-3 mimetic compound ABT-737 resulted in synergistic cytotoxicity, and the melanoma cell lines death associated with apoptosis (Risberg et al., 2011). Exotoxin A cloned with an anti-CD133 scFv reactive (dCD133KDEL) specifically kills CD133+ tumor initiating cells and can arrest the proliferation of head and neck squamous carcinoma cells *in vitro* and *in vivo* (Waldron et al., 2011). A fusion protein composed of circularly permuted IL-4 and a truncated form of exotoxin (PE) [IL-4(38-37)-PE38KDEL] effectively kills most multidrug-resistant tumor cells including lung carcinoma, breast carcinoma, and multiple myeloma (de Jong et al., 2003).

Diphtheria toxin

Diphtheria toxin (60 kDa, 538 amino acids) is exotoxin secreted by *Corynebacterium diphtheria* strains, which have the capacity for its production as a result of infection with bacteriophage B. The gene that encodes diphtheria toxin (tox) is present in some corynephages, and diphtheria toxin is only produced by *C. diphtheriae* isolates that harbor tox+ phages (Holmes, 2000). The toxin is composed of subunits A and B. The A subunit terminates host cell protein synthesis by inactivating elongation factor-2 (EF-2) preventing cell protein synthesis, thus leading to cell death. The B subunit contains receptor-binding region and a translocation region (Murphy, 2011). Diphtheria toxin (DT) has shown anticancer activity in both experimental models and humans. However DT has also adverse effects, therefore, DT is used in anti-cancer therapy in conjunction with other substances. Denileukin diftitox (DD; Ontak) is a recombinant DNA-derived cytotoxic protein composed of the enzymatically active domain (fragment A and a portion of fragment B) of the diphtheria toxin, followed by sequences of human interleukin (IL)-2 (Bacha et al., 1998). This molecule is used in treatment of chronic T cell lymphoma (CTCL) expressing CD25. Currently denileukin diftitox is in phase III studies (Duvic et al., 2013). DTAT is DT-based immunotoxin targeting tumor vascular endothelium. *In vitro*, DTAT has shown a potent antitumor activity against uPAR-expressing glioblastoma cells (U118MG, U373MG, and U87MG) and human umbilical vein endothelial cells. *In vivo*, DTAT caused a significant regression of small U118MG cell-induced tumors in mice (Vallera et al., 2002). Tf-CRM107 is a conjugate of human transferrin (Tf) and a genetic mutant of diphtheria toxin (CRM107) that lacks native toxin binding. Tf-CRM107 is targeting malignant brain tumors. The studies showed potent antitumor activity in a phase I clinical trial, in which 60% of patients showed a >50% reduction in tumor volume (Laske et al., 1997). Cross-reacting material 197 (CRM197) is the nontoxic mutant of diphtheria toxin. CRM197 commonly acts as an immunological adjuvant, or as an inhibitor of heparin-binding epidermal growth factor. The studies showed, that CRM197 blocked growth, reduced angiogenesis and induced apoptosis in human adrenocortical carcinoma (Martarelli et al., 2009). The combination of CRM197 plus doxorubicin enhanced cytotoxicity in a T-cell acute lymphoblastic leukemia cell line (Kunami et al., 2011) and that the combination with cisplatin inhibited growth and led to apoptosis of glioma cells (Wang et al., 2012).

Pep27anal2

Pep27anal2, is an analogue of Pep27 (3.3-3.6 kDa, 27-30 amino acids), the signal peptide produced by *Streptococcus pneumoniae*. It initiates death program of *S. pneumoniae*, and also has antimicrobial properties (Sung et al., 2007). Lee et al. synthesized an analog of Pep27 peptide, called Pep27anal2, composed of 27 amino acids. Obtained analog Pep27anal2, proved to be more hydrophobic molecule compared to the parent peptide. Lee et al. using the MTT assay, demonstrated a reduction of proliferation of leukemia cells (AML-2, HL -60, Jurkat), gastric cancer cells (SNU-601) and breast cancer cells (MCF-7). At the same time it was found that Pep27anal2 penetrates the cell membrane, and then induces caspase-independent and cytochrome c-independent apoptosis. Authors suggest that an important role in membrane permeabilization and in antitumor activity plays a hydrophobicity of peptide (Lee et al., 2005). Huang et al. believe that the hydrophobicity of the peptides plays a crucial role in the mechanism of action against cancer cells, which should be taken into account in the design of potential anticancer peptides (Huang et al., 2011).

Entap

A new, recently discovered anticancer peptide is Entap - Enterococcal anti-proliferative peptide (6.2 kDa), produced by clinical strains of *Enterococcus* genus bacteria (Szkaradkiewicz et al., 2012; Karpiński, 2012). In *in vitro* experiments strong anti-proliferative activity of Entap was demonstrated on human neoplastic cells, including gastric adenocarcinoma cells (AGS), uterine cervix adenocarcinoma cells (HeLa), mammary gland

adenocarcinoma (MDA-MB-231), prostate carcinoma (22Rv1) and colorectal adenocarcinoma (HT-29). Anti-proliferative activity of Entap in cancer cells is manifested by inhibition of their cell cycle at the phase of G1 and by induction of autophagous apoptosis. In ultrastructural studies of cancer cells exposed to Entap traits of degradation and autophagous apoptosis were detected: cytoplasm contained numerous vacuoles and structures corresponsing to autophagosomes and autolysosomes. Entap manifests thermal stability and high content of hydrophobic amino acids, which determines unique character of its molecule (Karpiński, 2012; Karpiński et al., 2013).

Conclusions

A lot of antitumor peptides, including some of bacterial peptides are characterized by low molecular weight and hydrophobicity. These features appear to be important for the penetration stage of these peptides into tumor cells, the surface of which differs in some of the characteristics from normal cells. Bacterial peptides are a specific group of anticancer drugs, now widely studied. Some of these are in clinical development what gives us hope for their pharmacological use in cancers treatment.

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