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Azurin: A Hardcore Anti-Cancer Agent

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A Review Article

ABSTRACT

Cancer is a life-threatening disease which is emerging as a major problem in the field of medicine. Different methods are being used to eradicate cancer including chemotherapy, radiotherapy, immunotoxins, and drug therapy but these treatments cause various side effects to the cancer patients, as they not only target cancer cells but also cause harm to normal body cells. At present some live bacteria, bacterial products, and bacterial proteins are used to treat cancer. One of the amazing bacterial protein azurin can work as an anti-cancerous agent which only target cancerous cells and do not cause any harm to healthy cells and thus minimize the risk of adverse side effects. The interaction of azurin with P53 (tumor suppressor protein), Eph/ephrin receptor and Cadherin enhances the activity of azurin by inducing apoptosis in cancerous cells, disrupting signaling among cancerous cells and inhibiting the growth of cancerous cells as well as suppressing the activity of tumor cells. This review article focuses on the activity of the bacterial protein azurin as an anti-cancer agent and also the properties of azurin which makes it one of the preferable anti-cancerous agent over the other cancer treatments like chemotherapy and radiotherapy.

Keywords: Microbial peptides, Cancer Drug, Azurin, Cancer therapy

INTRODUCTION

Cancer is considered a major disease in the world that can cause death (Ferlay *et al*., 2015). This disease is caused due to the activation of oncogenes and/or deactivation of tumor suppressor genes leading to uncontrolled cell division and inactivation of apoptotic events. Mutations, chromosomal translocations or deletions and deregulated expression or activity of signaling pathways are the mechanisms that are involved in the genetic and cellular changes which ultimately leads cancer. Epigenetic changes can cause cancer due to its role in the generation of cancer cells and the initiation of carcinogenesis (Sarkar *et al*., 2013). Every year cancer is identified in loads of people globally, and a lot of them die because of cancer. This problem increasing due to rapid population growth, and the reaction of oncologists, policy makers and general wellbeing experts and specialists (Bernardes *et al*., 2010). According to WHO (World Health Organization) projections, in 2030 about 11.4 million people are expected to die from cancer (Ferlay *et al*., 2015).

Main kinds of cancers include benign tumors and malignant cancers (Sarkar *et al*., 2013). Malignant and benign tumors are serious problems of the liver. Usually, benign tumors in the liver are cysts, focal nodular hyperplasia, adenoma, fatty infiltration, cavernous hemangioma, regenerative nodules and some unusual lesions like a liver abscess and angiomyolipoma. Malignant tumors cause metastases in the liver which include colorectal, gastric, pancreatic, hepatocellular carcinomas, cholangiocarcinoma derived from epithelial cells, lung and breast carcinomas (Xia *et al*., 2015). Cancer is also categorized by organ where it occurs, for example, Lung tumor, Breast tumor and Colorectal tumor etc. In 2012, the most analyzed sort of tumors was lung (1.82 million),

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bosom (1.67 million) and colorectal (1.36 million) (Ferlay *et al*., 2015).

Drugs used to treat cancer called anti-cancer drugs. As this disease involved in the deregulation of mammalian cell differentiation and growth it can be treated either by surgery, radiation or chemotherapy as well as by medication. Two types of drugs are being used in chemotherapy which includes small molecule drugs (tyrosine kinase inhibitors) and humanized proteins (monoclonal antibodies). Tumor medications, neglect to accomplish a total disease abatement and also cause other problems these medications include chemotherapy and radiotherapy. The advancement of numerous new methodologies for tumor treatment, for example, the utilization of live or lessened microorganisms to defeat this issue (Bernardes *et al*., 2010).

Over the remote past period, a fact of instinctive relapse of tumors linked to bacterial infections has been realized. In the 1890s an American doctor Coley observed the connection between bacterial infection and cancer relapse and discovered vaccine for cancer known as "Coley's toxin". After this research, interest develops to use live bacteria and their cleansed by-products as anti-tumor drugs. In 1909, William Coley used bacterial culture supernatants of *Streptococcus pyogenes* and *Serratia marcescens* to treat malignant cancer patients. Out of 1200 patients, 30 healed completely (Nguyen and Nguyen, 2016). Immunotoxins proved better than chemotherapy and radiation because in immunotoxins combo of toxin and antibody target specific proteins are used which are only present on tumor cell's surface. New tumor markers are found by which immunotoxins target tumor cells with high specificity, and low toxicity is employed (Allahyari *et al*., 2017). Until now it is exhibited that the factor in charge of this restorative impact expanded Tumor Necrosis Factor-α (TNF-α) discharge in the patient's body (Karpinski & Szkaradkiewicz, 2013).

Numerous reports have shown that microorganisms can replicate on tumor locations under hypoxic conditions (low concentration of oxygen) and can stimulate the host's immune system during the infections and blocked the cancer progression (Yamada *et al*., 2002). *Mycobacterium bovis* was used in the medication of bladder tumor in 1976 (Elkabani *et al*., 2000). It was demonstrated that the infection with bacterial pathogen agents activates macrophages and lymphocytes, producing cytotoxic agents with anticancer properties (Yamada *et al*., 2002). But sometimes its use may also cause some diverse effects (Paglia *et al*, 1997; Dang *et al*., 2001). The purified products of bacteria such as bacterial toxins, proteins and enzymes are useful in cancer treatment (Bernardes *et al*., 2010; Yamada *et al*., 2002). Various bacterial proteins and peptides are involved in cancer treatments such as weakened *Salmonella, Bifidobacterium* and *Clostridium* (Taniuchi *et al*., 2005). Azurin is a small (14 kDa; 128 amino acids) water-soluble protein secreted by *Pseudomonas aeruginosa* and used as an anti-cancerous agent against various types of cancers. It consists of one α-helix and eight β-sheets, forming a β-barrel motif and contains a hydrophobic patch (Karpinski and Szkaradkiewicz, 2013; Van de Kamp *et al*., 1990; Yamada *et al*., 2005; Fialho and Chakrabarty, 2012; Bernardes *et al*., 2013; De Rienzo *et al*., 2000).

Synthetic compounds as anti-cancerous agents

Various anti-cancerous synthetic drugs are being employed to cure different types of cancers, but these drugs cause various other side effects on the patient's body. ABVD is a synthetic drug which is commonly used to treat cancer, but it can cause several other problems in patients like increased risk of getting an infection, breathlessness and pale face, hair loss, loss of appetite, inflammation of lungs etc. (Keel *et al*., 2012). Similarly, Affinitor is used to treat breast, lung and pancreatic cancer can cause oral ulceration, renal failure and infections. BEP is used to cure ovarian and testicular germ cell tumor, but it can also cause side effects like bruising and bleeding, anemia, tiredness, hair loss, sore mouth, nail changes, hearing changes (macmillan). Etopside is used to cure lung cancer, and it causes side effects like hair loss, anemia, skin and nail changes, urination changes, pain, nausea and vomiting. Table 1 showed synthetic drugs approved by FDA/NIH as anti-cancerous drugs and are commercially available which are provided by various companies.

Table 1: Some anti-cancer drugs including the combination treatment

Medicines	Use to treat
ABVD	Hodgkin lymphoma
Afinitor, BEP	Breast cancer, Pancreatic cancer, Lung cancer, Ovarian germ cell tumors, Testicular germ cell tumor
Busulfan	Chronic myelogenous leukemia
CAPE-OX	Colorectal cancer
CAF	Breast cancer
Decitabine	MDS
Doxil	AIDS-related Kaposi sarcoma, multiplemyelo-ma, ovarian cancer
Etoposide	lung tumor , testicular tumor, Bosom cancer, Osteoporosis
Evista	Lung cancer, Breast cancer, Head and neck cancer, Acute lymphoblastic leukemia
Folex ICE	Hodgkin lymphoma, Nonhodgkin lymphoma

Natural products as anti-cancerous agents

Various naturally occurring compounds have shown anti-cancerous activity. These natural compounds are present in plants as well in microorganisms including fungi and bacteria (Kinghorn *et al*., 2016). Fungi produce several compounds such as cotylenin, which suppress the growth of tumor cells, cytochalasins which effects the movement of malignant tumor cells, alternethanoxins inhibits the growth activity of myeloma cells (Evidente *et al*., 2014). Plant compounds which are used to treat cancer are polyphenols, group of flavonoids, tennis, resveratol and gallacatechins. Polyphenols are capable of degrading DNA while other compounds such as brassinosteriods develop disease resistance and tolerance. There are also plant-derived drugs which are used to treat cancer like paclitaxel which blocks mitosis and induces apoptosis. Vincristine, vinblastine, vindesine and vinflunine are microtubule inhibitor and have pro-apoptotic properties. These drugs also induce cell cycle arrest. Roscovitine inhibits the cyclin-dependent kinase and also stop cell cycle (Greenwell & Rahman, 2015). Various approved plants products which are currently used for cancer treatment are shown in table 2.

Bacteria have naturally occurring proteins which are being used to treat cancer (Kumazaki *et al*., 2013). Yeast can also be genetically modified to produce anti-cancerous agents. Effects occurring in mutant yeast cells are used to determine the genetic defect in a cancer cell (Bjornsti, 2002). Staphylococcal superantigens are bacterial proteins, which have the capacity to bind to eukaryotic receptors which are overexpressed in cancer (Bernardes *et al*., 2010). Idarubicin is a microbial protein which binds to DNA template and breakdown it in leukaemia cells thus inhibits the activity of DNA polymerase (Fukushima *et al*., 1993). Bleomycin is a glycopeptide antibody which binds to the guanine cytocine rich part of the DNA. Its cytotoxicity is specific to G_0 phase (Dorr, 1992). Table 3 describes various microbial products which are being employed to cure various types of cancers.

Table 2: Anti-cancerous microbial compounds

Group	Examples
Aromatic polyketides (anthracyclines)	Daunorubicin, Valrubicin, Doxorubicin, Darubicin, Epirubicin, Pirirubicin, Amrubicin
Glycopeptides Non-ribosomal peptides Anthracenones	phleomycin, Actinomycin D, Streptozotecin, Mithramycin, Pentostain Bleomycin
Quinones Polyketides Indolocarbazoles Polyketides	Glycosides rebeccamycin, Enediynes calichaemycin, Macrolide lactones epotihilones, Mitosanes mitomycin C Ixebepilone
Nucleosides Halogenated compounds	2`-deoxycoformycin (pentostain) salinosporamide A

Table 3: Some endorsed plant-determined hostile to tumor mixes (Bermudes *et al*., 2002)

Compounds name	Plant source
Vinblastine (velban)	Madagascar periwinkle, *Catharanthus roseus*
Vincristine (on covin)	Madagascar periwinkle, *Catharanthus roseus*
Etoposide	Podophyllum peltatum
Teniposide	Podophyllum peltatum
Taxol(paclitaxel)	Taxus brevifolia
Vinorelbine	Madagascan periwinkle, *Catharanthus roseus* L
Taxotere	Taxus brevifolia
Camptothecin	*Camptotheca acuminate*
Topotecan	*Camptotheca acuminate*
Irinotecan	*Camptotheca acuminate*

Bacterial proteins and peptides as anti-cancerous agents

Cancer therapies (chemotherapy, radiotherapy, drug therapy) may cause many problems like the resistance of tumor cell and sometimes decreases its ability to eliminate micrometastases. So there is a need to explore more ways to cure the disease in a better way. Various bacterial proteins and peptides are currently proved as an efficient way to combat cancer. Soluble factors of *Salmonella*, *Clostridia*, and other anaerobic bacteria such as enzymes, secondary metabolites, proteins or peptides and toxins, may act on cancer cells as anticancer agents (Yamada *et al*., 2002; Bernardes *et al*., 2010; Dang *et al*., 2001).

In late-nineteenth-century, bacteria were discovered as anticancer agents. However, tumor patients after being infected with bacteria, develop bacterial infections at that time (Chakrabarty, 2003). Recent researches revealed that this problem could be reduced by using engineered bacteria with low infection capabilities or other bacterial products and also can target and kill just tumor cells and does not cause any harm to normal cells (Bernardes *et al*., 2010). Various bacterial proteins are discovered as an anticancer agent to restrict the activity of cancer cells leading to the death of a cancer cell or disrupting its signaling process for the production of cancer cells. *Mycobacterium bovis* Bacillus Calmete-Guerin (BCG) is one of the live bacteria used as the anti-cancerous agent. Its application is in bladder tumor, where a total reaction is given in around 80% of the patients(Cheadle and Jackson, 2002).

Another bacterium used in cancer treatment is *Listeria monocytogenes*. It is an intracellular bacterium, suitable for tainting phagocytic and non-phagocytic cells (Camilli *et al*., 1993; Rothman *et al*., 2010). After contamination with Listeria, a noteworthy safe reaction is instigated to clear the living being (Paterson and Maciag, 2005). Various live constricted L. monocytogenes strains to communicate viral and tumor antigens as combination proteins delivered amid the most recent quite a long while are: HPV-16 E7 (Gunn *et al*., 2001), Her-2/neu (Seavey *et al*., 2009; Singh *et al*., 2005), HMW-MAA (Maciag *et al*., 2008), influenza NP (Ikonomidis *et al*., 1994) and PSA (Shahabi *et al*., 2008;Wallecha *et al*., 2009). These recombinant strains cause CD4+ and CD8+ T cell responses in mice (Gunn *et al*., 2001; Singh *et al*., 2005). L.monocytogenes is not just a trigger of a potential insusceptible reaction and an effective antigen conveyance framework yet, for the most part, another promising anti-cancer operator(Camilli *et al*., 1993; Rothman *et al*., 2010). Various bacterial proteins and peptides which are used as anti-cancerous agents are shown in figure 1.

Azurin: a bacterial protein as an anti-cancerous agent

Azurin is a bacterial protein which is produced by *Pseudomonas aeruginosa*. Azurin works as an anticancer agent, and it has the ability to bind to numerous targets intracellularly and extracellularly in mammalian cells. Azurin enters into the human cancer cells through the amino acids (50 -77) of the protein (peptide p28) (Yamada *et al*., 2005). Azurin and p28 enter the plasma membrane through the caveolae-mediated endocytic pathway which leads to endosomes, lysosomes, and the Golgi associated with caveolae (Taylor *et al*., 2009). P28 can also enter human umbilical vein endothelial cells (HUVEC), linked with caveolin-1, which affects the motility and migration of HUVEC (Mehta *et al*., 2011).

Recently it was demonstrated that two internal breast cancer cell lines were treated with azurin, leading to unique changes in the genes

ultimate apoptosis. Azurin up-regulates the genes associated with vesicle-mediated transport, endosome formation and membrane organization, common for both normal as well as cancerous cell lines (Bernardes *et al*., 2014].

Signaling molecules, including, receptor and non-receptor tyrosine kinases, G-protein subunits, small GTPases and endothelial nitric oxide synthase bind to caveolin-1 through its caveolin scaffolding domain (Williams and Lisanti, 2004; Staubach and Hanisch, 2011). The caveolin-1 level is linked with lung, breast, prostate, and their lymph node metastases, increasing the possibility that caveolin-1 may act as an oncogene (Ho *et al*., 2002).

Sources of Azurin

Azurin is derived from *Pseudomonas aeruginosa* which is a gram-negative, rod-shaped bacterium (Pozdnyakova *et al*., 2001). *Pseudomonas aeruginosa* is a nosocomial, and is responsible for 10 - 15% nosocomial infections which are hard to treat because of the antibiotic resistance of the species and can adopt resistance during cancer treatment (Pechere *et al*., 1999). Pathogenesis in *P. aeruginosa* target the extracellular matrix and facilitates adhesion, modulate or disrupt host cell pathways. *P. aeruginosa* provokes an inflammatory response during the infectious process (Alhazmi, 2015). This species is naturally resistant to many drugs (Strateva and Yordanov, 2009). Its essential proteins are F, H2 and I that are large in number (Hancock *et al*., 1981).

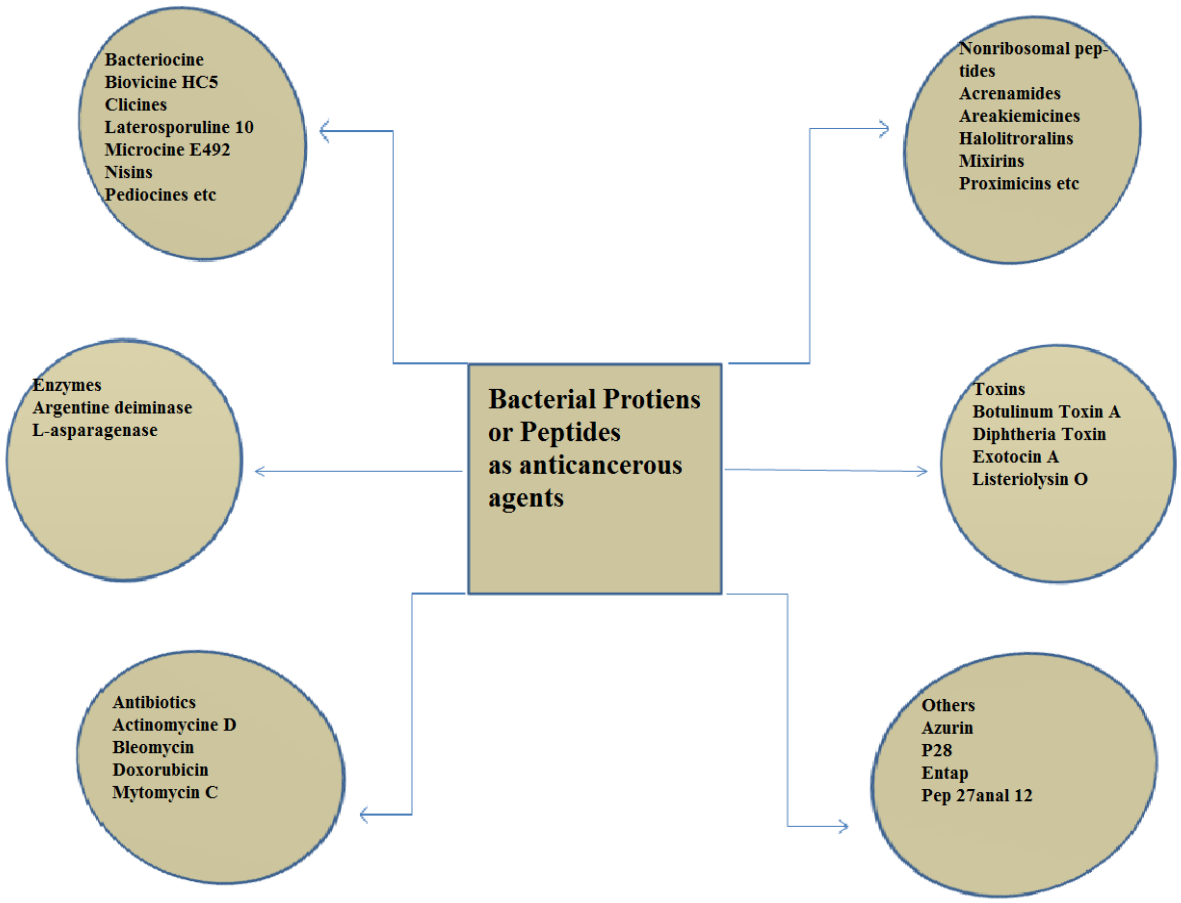

Fig. 1: Bacterial proteins and peptides which are used to cure cancer

Structure of Azurin

Azurin is a single peptide chain along with 128 amino acids and is blue due to the presence of copper ions in it (Pozdnyakova *et al*., 2001). The azurin protein is the individual from a group of copper containing redox proteins called cupredoxins, created by different oxygen-consuming microscopic organisms going about as operators of electron exchange (Fialho & Chakrabarty, 2012). It is one of the simplest copper proteins, having one copper atom in it (Van Pouderoyen *et al*., 1997). Azurin consists of one α-helix and eight β-sheets and forms a β-barrel motif. Azurin is linked to two protein redox centers: T1 blue-copper ion coordinated with amino acid residues and a disulfide bridge located at the opposite end of the molecular structure (Farver *et al*., 1982).

Salient features of Azurin

Azurin is involved in the transport of electrons during the respiration (Yamada *et al*., 2002) and serves as an electron carrier protein during respiration (Hoitink *et al*., 1992), transporting an electron between cytochrome (cyt) c-551 and cyt oxidase in bacterial respiration process (Parr *et al*., 1977; Silvestrini *et al*., 1982). Azurin cause apoptosis in cancer cells (Punj *et al*., 2004). Azurin is also produced by other bacteria rather than *Pseudomonas aeruginosa* (De Rienzo *et al*., 2000). Azurin increases the intracellular levels of Bax and p53 in the nucleus, which triggers the release of mitochondrial cytochrome c in the cytosol. It activates the caspase cascade (including the caspase-7 and caspase-9) starting the apoptosis (Punj *et al*., 2004).

Mode of action of Azurin

Cells in healthy tissues divide when they receive signals for growth, or growth factors, from other cells. These are detected by growth factor receptors in the ECM (extracellular matrix). Tumor cells do not depend on exogenous growth factors, due to endogenous production of mitogenic factors, which they secrete into the ECM (Leber *et al*., 2009). Tumor cells may over-express the receptor tyrosine kinases (RTKs) on the surface or produce structurally changed RTKs that activates the signals even in the absence of mutagenic factors. In addition, various proteins which detect and repair DNA damage or the metabolism in a normal cell, are mutated in cancer cells (Yamada *et al*., 2002).

Azurin work as an anticancer agent. There are various patents about the use of azurin in cancer therapies, and azurin has shown positive outcomes (Fialho & Chakrabarty, 2012). There are two modes of action of azurin to cancer cells which have been described: one through p53 and the other by action through transmembrane proteins, such as the Eph receptors family or P-cadherin.

Azurin and p53

P53 is an inducer of apoptosis in cells and cause apoptosis in cancer cells (Yamada *et al*., 2002). It is a 393-residue tumor suppressor protein. P53 protein can transactivate genes (arrest) involved in cell cycle and interact with proteins for apoptosis (Vogelstein *et al*., 2000). This is done by up-regulation of p21 (Harris *et al*., 1996). It can be divided into three domains: the N-terminal domain (NTD); a DNA-binding domain (DBD); and C-terminal domain (CTD) (Okorokov *et al*., 2009). It plays a role in cellular processes, including transcription, DNA repair, cell cycle control and apoptosis. The azurin forms a stable complex with p53 (Taranta *et al*., 2008) which lead to its increase intracellular level in cytosolic, mitochondrial, and nuclear fractions (Yamada *et al*., 2002). MDM2 (Mouse double minute 2 homolog) binds to the 18-23 residues of p53 transactivation domain, changing it from unstructured to α-helical, inhibiting p53 transcriptional activity, its nuclear export and results in its degradation. In other words, it stabilizes p53 and maintains its function (Gabellieri *et al*., 2011).

Azurin has the ability to bind with specific domains of p53 in multiple possible ways. However, the binding regions of both proteins are not properly identified. It was demonstrated that azurin binds to the transactivation domain of p53 and targets the copper near the tryptophan (Apiyo *et al*., 2005). Azurin can also bind to the DNA-binding domain (DBD) of p53, increasing its intracellular levels (Yamada *et al*., 2002). However, azurin does not bind to the MDM2-binding site (Yamada *et al*., 2009).

The azurin peptide p28 has infiltration, and anti-proliferative impact on human bosom growth cells intervened by p53, hence the p53 increase in response to p28. p28 binds within amino acids 80 to 276 of the p53 DBD, along with the interaction between p53 and ubiquitin ligases, decreasing ubiquitination and cause degradation of p53. P28 activates p21 and p27 and inactivates the CDK2-cyclin A complex, causing a cell cycle arrest in breast cancer cells. P28 effectively enters into the human breast cancer cell lines MCF-7, ZR-75-1, and T47D through a caveolin-mediated pathway. This lessens degradation of p53 and may give a unique series of cytostatic and cytotoxic chemotherapeutic agents or drugs (Yamada *et al*., 2009). Binding of azurin with p53 and p28 is shown in figure 2.

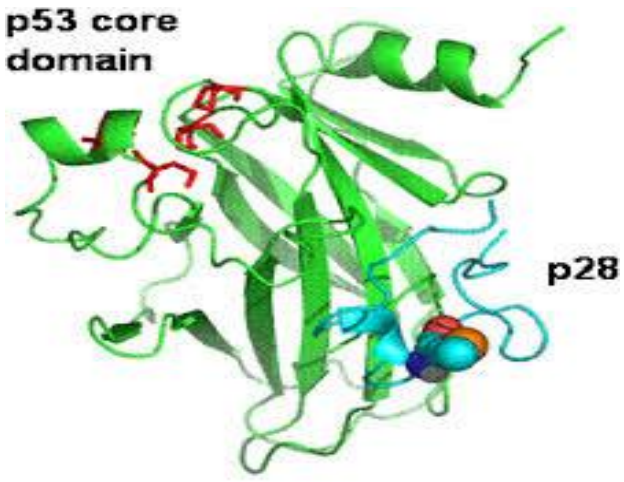

Fig. 2: Displaying the cooperation between the p53 DNA-restricting space and the p28 peptide piece of Azurin (Santini *et al*., 2011).

Azurin and Eph/ephrin receptor

Azurin also showed the capability to bind with various Eph/ephrin receptor tyrosine kinases, which is an extracellular receptor protein up-regulated in many tumors. This binding result in EphB phosphorylation, resulting in disrupting of cell signaling and cancer growth (Chaudhari *et al*., 2007). Azurin likewise focuses on a cell expansion pathway intervened by the EphB2 tyrosine kinase. The action of azurin is through transmembrane proteins such as Eph receptors family or P-cadherin. Eph/ephrin interaction initiates many cellular signaling processes, like proliferation, migration, invasion and angiogenesis (Blits-Huizinga *et al*., 2004). These processes result in cancer progression and up-regulation in various tumors (Nakada *et al*., 2004). Azurin is structurally similar to ligand ephrinB2, which binds tyrosine kinase EphB2 to start cell signaling. This binding to EphB2 is overexpressed in many types of cancer, which prevents the tumor progression. Azurin binds to the EphB2-Fc receptor with high affinity, and inhibits the ephrinB2-mediated autophosphorylation of the EphB2 tyrosine residue, affecting cell signaling and inhibiting cancer cell growth (Chaudhari *et al*., 2007).

Azurin and Cadherins

Recently it was discovered that azurin treatments in breast cancer cells are related to P-cadherin and FAK/Src signaling. Azurin decreases breast cancer cells motility and invasion, by reducing the amount of P-cadherin in the cell (Bernardes *et al*., 2013). Cadherins are cell-cell bond glycoproteins that shape calcium-subordinate intercellular intersections (Gumbiner *et al*., 1993; Paredes *et al*., 2012). During tumor progression, these molecules are changed. E-cadherin forms junctions, which are required for the initiation and maintenance of a homeostatic intercellular space and cell-to-cell interaction. E-cadherin also recognizes signaling molecules and serves as docking sites for vesicles (Nelson *et al*., 2003).

Although, P-cadherin involved in cell-to-cell adhesion, its expression is restricted to specific areas of epithelial tissues (Hirai *et al*., 1989). E-cadherin is a tumor suppressor leading to the activation of specific signaling pathways and interaction with various other molecules. One example of E-cadherin linked with the signaling of cytoskeletal network, regulated by the action of the individuals from the Rho group of little GTPases (Rho, Rac and Cdc42) that involve filipodia, lamellipodia and contractile forces to move the body of a migrating cell (Paredes *et al*., 2012). E-cadherin co-localize with specific receptor tyrosine kinases (RTKs) to basolateral areas of epithelial cells and form multicomponent complexes with them (Pece *et al*., 2000).

The p-cadherin expression has a remarkable role in the prognosis of invasive breast cancer that also maintains E-cadherin expression and acts as a biomarker of poor prognosis in E-cadherin positive breast carcinomas. Overexpression of P-cadherin is caused by the cytoplasmic accumulation of one of the catenins, p120ctn (Taniuchi *et al*., 2005). P-cadherin overexpression in wild-type E-cadherin breast cancer cells increases cell invasion, motility and migration.

The P-cadherin initiates the secretion of pro-invasive factors, such as MMPs, which then lead to P-cadherin ectodomain cleavage. This soluble P-cadherin fragment (called sP-cad) is released to the extracellular surface and is responsible for in vitro invasion of wild-type E- and P-cadherin expressing cells as well as in noninvasive cells (Ribeiro *et al*., 2010).

Recently it was discovered that the phenotype of P-cadherin-overexpressing breast cancer cells was reduced by azurin, as well as the levels of sP-cad were reduced and it does not affect E-cadherin levels. Azurin decreases the invasion of two P-cadherin expressing breast cancer cell models, linked with a decrease in the total P-cadherin (Bernardes *et al*., 2013).

Azurin can target both P-cadherin and induces expression at the membrane level, along with signaling pathways linked with them. Azurin reduces the mammosphere forming efficiency of these cells in independent growth conditions (Bernardes *et al*., 2014).

This also decreases the level of FAK (Focal adhesion kinase; a protein tyrosine kinase that regulates cellular adhesion, motility, proliferation and survival in different sorts of cells) and Src (non-receptor tyrosine kinase; deregulated in many types of *cancer* and plays a role in tumor development, including adhesion, proliferation, invasion, survival, migration, and most importantly, metastasis, in multiple tumor types) without any changes in total FAK and Src protein levels (Bernardes *et al*., 2013).

Advantages of Azurin as an anti-cancerous drug

Azurin has the potential to act as an anticancer agent. Azurin entry in cancer cells showed no adverse side effects as observed in vivo studies (Yamada *et al*., 2005; Cho *et al*., 2011; Warso *et al*., 2013). As mentioned above, this protein has high-affinity interactions with different inconsequential mammalian proteins applicable in cancer, giving it as a natural scaffold protein, an important characteristic of this protein thus trigger resistance by the cells (Fialho *et al*., 2007).

Another advantage of azurin is that it is a water-soluble molecule with a hydrophobic patch, thus helping in tissue penetration and clearance from the bloodstream (Van de Kamp *et al*., 1990). Azurin can be easily over-expressed in *Escherichia coli*, which makes the process of production very cheap and cost-effective (Bernardes *et al*., 2013), expression may occur in different vectors, including some human cell types and this makes azurin an attractive molecule to be used in cancer therapy.

The use of p28 showed significant evidence that there is no toxicity or immune response in the patients with solid tumors p53+/+, on which No Observed Adverse Effect Level (NOAEL) and Maximum Tolerated Dose (MTD) were accomplished (Warso *et al*., 2013). Thus azurin has low immunogenicity, a non-antibody recognized protein and therefore is not susceptible to immune attack, even though it is a bacterial protein. p28 is safe and well tolerated in children with progressive CNS malignancies according to recent studies (Lulla *et al*., 2016).

There are various domains in azurin with anticancer property (Chaudhari *et al*., 2007) that give better viability and make azurin less vulnerable to opposition improvement gave absence of lethality of azurin in creatures and in addition tumor patients can be illustrated, as improved the situation p28 (Fialho *et al*., 2012).

Weekly or bi-weekly injection of azurin in vulnerable people, for example, women with family history of breast or ovarian cancers and along with diagnosed BRCA1/BRCA2 mutations, is an efficient way to prevent and reduce cancer in such people. Besides these various other pathways of azurin for cancer treatment, like oral are recently investigated (Chakrabarty *et al*., 2014).

The p28 can be combined with drugs like cargo proteins, facilitates their transfer to cancer cells which cannot enter in eukaryotic cells on their own (Yamada *et al*., 2005), or nanoparticle-loaded drugs can be surface coated with azurin to increase its efficiency. The azurin or its derived peptides act as diagnostic markers to locate or point out tumors inside the body and then moves towards cancer cells without causing any harm to normal cells (Chakrabarty *et al*., 2014).

Delivery of Azurin in host

The mechanism of entry for azurin into the cancer cells is still not fully understood. However, it enters in human cancer cells by the amino acids 50 - 77 of the protein (p28), which forms an α-helix with both a hydrophobic amino acids (50 - 66) and hydrophilic amino acids (67 - 77) (Yamada *et al*., 2005; Taylor *et al*., 2009). The peptide p28 was further linked to amino acids 50 - 67. This is responsible for the entry of azurin into human cancer cells, and it is called p18. p28, p18 and azurin enters into the plasma membrane and reaches late endosomes, lysosomes, and the Golgi related with caveolae. The lipid rafts were disrupted, by depletion of cholesterol, which inhibit the penetration of p18 and p28, directing that they penetrate the plasma membrane via caveolae endocytic pathway. This procedure is not reliant on film bound glycosaminoglycans neither on clathrins however N-glycosylated proteins may have a part at

any rate in the underlying strides of acknowledgement. These protein and peptides tie to growth cells with high affinity, suggesting that this protein/receptor complex localizes in the caveolae. P28 and p18 also penetrate the plasma membrane via a non-clathrin-caveolae–mediated process (Taylor *et al*., 2009). P28 enters human umbilical vein endothelial cells (HUVEC), colocalized with caveolin-1 and VEGFR-2 and hinders VEGF-actuated relocation, slim tube arrangement and neoangiogenesis in xenograft models (Mehta *et al*., 2011).

In the MCF-7/AZ.The pcad cell line, genes linked with different phenotype were changed as well as genes associated with the cell and biological adhesion (Bernardes *et al*., 2014). Overexpression of P-cadherin resulted in up-regulation of genes. Whereas while treating cells with azurin, results in a down-regulation of these genes and up-regulation of genes linked with apoptosis progression.

Azurin works as an anti-cancerous agent. It directly targets specific proteins present on cancer cells and does not cause any harm to normal cells. The interaction of the azurin with p53, p28, Epherin receptor and cadherins, enhances the anti-cancerous activity of azurin towards cancerous cells. Azurin minimize the adverse side effects normally caused as other cancer treatments.

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