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ENGINEERED *E. COLI* NISSLE 1917 PRODUCING AZURIN FOR TARGETED COLORECTAL CANCER THERAPY

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Abstract

Azurin is a redox-active bacterial protein with selective antitumor activity through stabilization of p53 and suppression of oncogenic signals. We designed *E. coli* Nissle 1917 to express and secrete azurin under the control of lacUV5 and P_vhb promoters with the pelB signaling peptide. Future work aims to explore synergetic effects of co-delivering azurin with additional pro-apoptotic proteins.

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths. The effectiveness of standard chemotherapy is limited by its systemic toxicity [1]. Protein-based therapeutics offers an alternative approach with higher specificity and lower side effects [2], but their clinical application is hindered by instability and delivery problems, especially with oral administration [2, 3]. A promising strategy is to use genetically modified bacteria to guide the delivery of antitumor proteins directly to the tumor area. The probiotic strain *Escherichia coli* Nissle 1917 (EcN) is able to selectively colonize colon tumors when administered orally [4]. Clinical studies have shown that EcN accumulates significantly more in colon tumor tissue than in healthy tissue [1, 4]. The EcN strain lacks virulence factors and is rapidly cleared from healthy organs because of its serum-sensitive LPS, thus the strain is nonpathogenic and highly safe [5]. These properties make EcN an attractive platform for targeted microbial therapy of colorectal cancer.

Azurin is a copper–containing protein (~ 14 kDa) isolated from *Pseudomonas aeruginosa*, which is considered a potent antitumor agent. It selectively penetrates cancer cells and induces their apoptosis. Azurin has demonstrated cytotoxicity against a wide range of malignancies, including melanoma, breast, lung, ovarian, and colorectal cancers [6]. Azurin engages with key tumor-suppressive pathways, for example, it forms a complex with the p53 protein, preventing ubiquitin-dependent degradation of this tumor suppressor. In addition, azurin inhibits angiogenesis by blocking signal transmission through VEGFR2 [7]. The combination of these effects leads to inhibition of tumor growth and regression, showcasing the capabilities of azurin.

In this work, we engineered an *E. coli* Nissle 1917 strain to produce and secrete azurin for targeted CRC therapy. We constructed plasmids carrying the mature azurin gene under the control of two types of promoters: (1) lacUV5 (with a Lac operator) for induced expression and (2) P_vhb (a promoter from the *Vitreoscilla* hemoglobin gene) for activity under hypoxic conditions typical of the tumor microenvironment. To ensure the secretion of the target protein outside the bacterial cell, its N-terminal region was fused with the pelB signaling peptide, which directs azurin into the periplasm subsequently outside the cell. We validated the expression of azurin in *E. coli* strain BL21(DE3) at 37 °C (using an expression plasmid based on pET303), observing a high level of protein synthesis. Experiments are currently underway to evaluate the effectiveness of azurin secretion by the EcN engineered strain and its cytotoxic effect on colorectal cancer cell cultures (HCT116, Caco-2).

Furthermore, we plan to investigate synergistic anti-cancer strategies through the combined delivery of azurin with other pro-apoptotic proteins. Such proteins include PUMA (p53-upregulated modulator of apoptosis), a member of the Bcl-2 family, a key initiator of the internal (mitochondrial) pathway of apoptosis [8], and MakA, a bacterial cytotoxin from *Vibrio cholerae* that selectively kills colorectal cancer cells by destabilizing lysosomes and suppressing the β-catenin signaling pathway [9]. We assume that the combined delivery of azurin with such pro-apoptotic factors will enhance the death of tumor cells by simultaneously affecting various mechanisms of apoptosis.

Local delivery of azurin using *E. coli* Nissle 1917 will allow targeted destruction of tumor cells in the intestine with minimal systemic side effects. Overall, our approach may open new avenues for developing safe and effective biotherapeutic strategies for the treatment of CRC.

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