REGULAR ARTICLES

Bacterial infection-mediated anticancer activity (BIMAc) – Revisiting the molecular mechanisms

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Abstract The anticancer activity demonstrated by genetically attenuated invasive Shigella flexneri contradicts the long-held understanding of bacterial infection-mediated anticancer activity (BIMAc), as a ‘by-stander effect’ caused by an immune response against any invading pathogen as a reason for tumour regression. Similarly, the selective tumouricidal effect by Salmonella A1 auxotrophic mutant in nude mice is another observation where the current theory fails. Considering these flaws, we set to re-examine the mechanisms behind BIMAc independent of immune response, on the basis of molecular understanding about the initial colonisation of gut epithelium by S. flexneri and its production of cell-cycle-inhibiting proteins called cyclomodulins. During infection, S. flexneri injects OspE effector protein into the gut epithelium. The resulting interaction of OspE with ILK prevents epithelial cell exfoliation and facilitates the pathogen’s colonisation of the gut. This interaction is also shown to enhance membrane retention of ILK in these infected cells. Correspondingly, another study reports the indispensable role of ILK in survival of cancer cells with supernumerary centrosomes by localising it to the centrosomes and clustering them into a bipolar spindle. Knockdown of ILK in these cells leads to apoptosis due to multipolar mitosis. From these cumulative facts we hypothesised that enhanced membrane retention of ILK in Shigella-infected cancer cells prevents localisation of ILK to centrosomes and provokes multipolar mitosis and therefore cell death in cancer subpopulations with supernumerary centrosomes. This interaction may also be metastasis suppressive, because of its inhibitory effect on the focal adhesion turnover of gut epithelium, which is quintessential for any form of cell migration. Apart from these, Shigella also encodes potent cell-cycle-inhibiting effector molecules such as cyclomodulins. The additive action of these cyclomodulins along with the OspE–ILK interaction may be considered as the reason behind the anticancer activity mediated by Shigella infection.

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Introduction

The beneficial effects of bacterial infection in a clinical scenario were first effectively exploited by William B. Coley, circa 1891. He administered a heat-attenuated mixture of bacteria called 'Coley’s toxin' to cure inoperable malignancies, of which the mixture of Streptococcus and Serratia marcescens was most effective. Despite the controversies surrounding the use of Coley’s toxin, he is regarded as the ‘Father of immunotherapy’ [1]. Apart from the seminal findings of Coley, the idea of using live-attenuated bacteria as anticancer agent was also established gradually [2]. Clostridium [3,4], Salmonella [5–7], and Shigella [8,9] are some examples of live-attenuated bacteria being pursued for their antitumour activity and their use as pro-drug delivery vectors. A tumour’s primitive angiogenesis and microenvironment making it vulnerable to a generic immune response like hyperthermia (which is provoked by repeated administration of Coley’s toxin or bacterial infection) is held as the predominant theory (cause) behind bacterial infection-mediated anticancer activity (BIMAc) [10]. In an earlier study, the anticancer activity of a genetically attenuated invasive Shigella flexneri was compared with a non-invasive mutant, using murine breast cancer model [8]. It was observed that the non-invasive mutant was unable to elicit any anticancer activity which is counter-intuitive to the current theory, which claims the immunogenicity of bacteria as a reason for the anticancer activity. In a separate study, the selective tumouricidal effect by a Salmonella A1 auxotrophic mutant in xenograft tumours in nude mice [5,6] was observed. This further questions the veracity of the claim that immunogenicity is the main mechanism of BIMAc, since these mice are devoid of a functional immune system. Considering these caveats in the present understanding, we set out to re-examine the mechanism(s) behind BIMAc based on the recently elucidated molecular understanding about Shigella’s initial colonisation of gut epithelium [11] and effects of its cell-cycle-inhibiting cyclomodulins [12,13].

Initial colonisation by S. flexneria of gut epithelium by targeting integrin linked kinase (ILK) with its outer shigella protein E (OspE) protein

Rapid exfoliation of gut epithelium acts as an innate defence against colonisation by any pathogen [14]. S. flexneri, a pathogenic Gram-negative bacterium responsible for Shigellosis, overcomes this innate defence mechanism by inhibiting the epithelial cell exfoliation. It does so by injecting its OspE protein into the epithelial cells through its type three secretory system (T3SS). The injected OspE docks with ILK of the host, which inhibits focal adhesion turnover, and, thus, the subsequent epithelial exfoliation. It was shown that OspE–ILK docking does not result in the inhibition of its enzymatic activity in an in vitro kinase assay, but the steric hindrance caused by OspE dockering with ILK inside the cell affects its activity which is reflected by the significant reduction in phosphorylation of its downstream substrate like Paxillin and focal adhesion kinase, whose phosphorylation in turn is essential for focal adhesion turnover. Apart from this, OspE–ILK docking enhances the membrane retention of ILK [11].

Role of ILK in inhibiting the multipolar mitosis

The presence of supernumerary centrosomes aids the process tumourigenesis in various ways [15–17]. However, the status of a cell containing supernumerary centrosomes is in jeopardy due to the inherent risk of multipolar mitosis which can occur, thus leading to cell death [18]. ILK plays a vital role in preventing multipolar mitosis by clustering the supernumerary centrosomes to form a bipolar spindle resulting in a normal mitotic division. Although ILK does this by regulating the phosphorylation of transforming acidic coiled-coil-containing protein 3 (TACC3), which is required for centrosomes clustering through aurora-kinase A, the physical localisation of ILK to the centrosomes is indispensable for this process [19].

Cyclomodulins

These are a class of effector molecules produced by the bacteria which have the ability to modulate (pro-proliferative or anti-proliferative) eukaryotic cell cycles [13]. S. flexneri encodes invasion plasmid antigen B (IapB) and cytolethal distending toxin (CDT) [20]. Both of these proteins have a cell-cycle inhibitory effect, out of which IapB accompanies OspE during its delivery through T3SS into the host cell and specifically targets the G2/M transition in cell cycle [12], while CDT induces cell-cycle arrest by provoking double-stranded DNA breaks [21,22].

Hypothesis

From the above facts we hypothesise that the enhanced membrane retention of ILK in Shigella infected cancer cells caused by OspE prevents the ILK localisation to centrosomes and provokes multipolar mitosis, thus leading to cell death in the subpopulation harbouring supernumerary centrosomes. This interaction may also result in suppression of metastasis, because of the inhibition of focal adhesion turnover on gut epithelial surface, which is necessary for any form of cell migration. Apart from these two modes of host–pathogen interactions, Shigella also encodes potent eukaryotic cell-cycle-inhibiting effector molecules such as IapB, CDT (collectively called cyclomodulins). We reason that the cumulative effects of aforementioned processes in the host–pathogen interactions are mechanistically responsible for BIMAc by S. flexneria (Fig. 1).

Evaluation of the hypothesis

We argue against the existing notion of immunogenicity-driven anticancer activity of bacteria by hypothesising that a specific set of molecular events involving multipolar mitosis, cell-cycle inhibition by bacterial cyclomodulins and by restriction of cell migration may additively be the reason for the observed phenomenon. To bridge the gap between our proposed hypothesis and its establishment as a theory, a number of experimental validations are required. Previous studies have already established the function and role of bacterial cyclomodulins [13,12,21,23] as cell-cycle inhibitors. The multipolar mitosis-inducing effect of OspE can be assessed expressing OspE in BT549 and MDA–MB–231 cell lines which are reported to possess high frequency of supernumerary centrosome-harbouring...
The cell migration inhibitory effect of OspE can be investigated using migration/invasion assay in the same context. However, these two experiments will only prove the role of OspE–ILK interaction in the context of proposed molecular outcome. To provide unequivocal evidence, a comparative assessment of the tumouricidal effect of OspE, IapB and CDT knockout mutants with its wild-type *S. flexneri* has to be performed. This proposed animal study has to be done in both immune-competent and compromised backgrounds, respectively, to determine the extent of contribution of immunogenicity-independent mechanism discussed here.

**Discussion**

The notion of using live-attenuated bacteria for anticancer potential has gradually evolved from the advent of Coley’s toxin[1] followed by the use of Clostridium novyi-NT [2], *S. flexneri* [7], Vnp20009 [6] and right to the development of highly efficient auxotrophic mutants of salmonella [4,5]. In spite of these advances, the underlying explanation for the infection-mediated anticancer activity is superficial, being attributed to a general immune response-propelled bystander effect on tumour mass [1], and the anaerobic nature of bacteria or auxotrophic mutations for its selective accumulation in tumour mass [4,5]. Though our hypothesis fits well for the explanation of the immunity-independent effects of bacterial infections and tumour regression, it does not account for the scepticism that cyclomodulins (especially CDT) may be cancer promoting [13,24]. However, all these circumstantial speculations have to be reviewed in depth since these studies were conducted in a system where normal physiological perturbation was associated with the occurrence of cancer, whereas the idea of using pathogenic bacteria against tumours already underscores the altered physiological conditions which exist in cancer. Our argument parallels the rationality of using any of the DNA-damaging drugs for the chemotherapy of cancer which would invoke different responses in homeostatic proliferating cells (side effects) [25], since the physiology differs in each case. Additionally, there is an intrinsic checkpoint on the systemic survival of pathogens in areas other than tumour mass by making use of genetically attenuated and auxotrophic mutant stains of these pathogens, enabling us with a better measure of modulating the clinical outcome of using these modalities [4,5,7].

**Conclusion**

To achieve a real bench to bedside translation for usage of these bacteria, it is necessary to dissect the molecular underpinnings of their antitumour activity. This extensive understanding is crucial for current medical standards and accelerating progress towards personalised molecular medicine. Hence, we speculate that the proposed hypothesis and its establishment as fact will be a gateway for further advances in the field of using bacteria as therapeutic modules for cancer therapy. Furthermore, cataloguing of the high-impact molecular interactions such as OspE, CDT and IapB, which are otherwise hidden under the complex crosstalk happening between host and pathogen in the context of cancer, will lead to a system where control and fine tuning of ‘BIMAc’ can be achieved by design of genetically altered and targeted mutants of above-mentioned bacteria pertaining to clinical perspectives.

**Conflicts of interest**

The authors declare they have no conflicts of interest. The authors state the employers (Institute of Genomics and Integrative Biology) had no role in the conception, discussion and writing of the manuscript.
Overview Box

First Question: What do we already know about the subject?
Bacterial infections in some cancer patients tend to suppress tumour growth. Hyperthermia or other immune responses against such bacteria are thought to be the factors responsible for BIMAC.

What does your proposed theory add to the current knowledge available, and what benefits does it have?
(A) The current hypothesis explains BIMAC at the host–pathogenic interaction level underlining specific molecular interactions, independent of the immune response.
(B) Further engineering bacteria for therapeutic applications based on our hypothesis will avoid the risk of employing or provoking any adverse immune reaction.

Third question: Among numerous available studies, what special further study do you propose for testing the idea?
Comparing the anti-cancerous efficacy of the wild-type virulent strain to the non-immunogenic mutants will provide a direct evidence to facilitate the establishment of the above hypothesis by clearly emphasising the contribution of immunogenicity of the bacteria in suppressing tumour progression to that of the proposed immune-independent molecular interactions.

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