Bioengineered Microbes in Disease Therapy

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Rahul Mehta

Abstract

The developments in biotechnology, genomics, proteomics, immunology, and metabolic engineering have provided with unprecedented applications of microbes to improve human health. Disease therapies are experimenting with new regimes to alleviate the problems of diseases. Bioengineered microbes present with ample opportunities to uniquely address the problem of ailments by increasing the length and breadth of the available options to tackle diseases. Microbial immunomodulation of the host, targeted killing of the tumor cells using microbes, and specific delivery of drugs into the host tissues are some of the examples of application of bioengineered microbes. Although history of the concept of possibility of using the microbes for tweaking the disease progression and therefore promoting human health is an old one, with recent research studies, the field has received a much needed impetus. In the future, the need is to establish more in vivo evidences for wide acceptance of therapies using bioengineered microbes as well as to demonstrate the extent of the possible horizontal transfer of such bioengineered genes in order to undertake its practical applications.

Keywords

Bioengineered microbes · Therapeutic · Immunomodulation · Tumor cells

R. Mehta (✉)
Department of Microbiology, DAV University, Jalandhar, India

S. G. Sharma et al. (eds.), *Microbial Diversity, Interventions and Scope*,
https://doi.org/10.1007/978-981-15-4099-8_8

8.1 Introduction

The relationship between humans and microorganisms has been well established and studied for over a century with microbes used in various applications for the benefit of human race (Paton and Paton 2012). The major areas in which microbes have been a key player include but are not limited to model organisms in laboratory (for development of molecular biotechnology and studying gene functions), industrial microbiology (development and production of newer products), and the pathogenic microbes (studying infections and potential way of controlling them). However, the discovery of advanced techniques in molecular biology studies, genetic tweaking, and immunology has brought unforeseen avenues to develop newer and better bioengineered microbes with multidimensional applications in disease therapy (Das et al. 2010; Chikazu D et al. 2017). Bioengineered microbes can be helpful in disease management and therapies (Yu H et al. 2011) in one of the following possible ways:

- Immunomodulation by probiotics (Culligan EP et al. 2009)
- Targeted delivery of drugs and gene therapy vectors with a higher site specificity in comparison with traditionally employed methods (Paton et al. 2012)
- Use of anaerobic bacteria to specifically target the killing of tumor cells (Roberts et al. 2014)
- Use of bioengineered microbes like *Salmonella* sp. to target specific viral mRNAs with RNase P (Al-Ramadi B.K. et al. 2009)

Thus, it becomes quite evident that bioengineered microbes have a variety of applications (Table 8.1) and they can significantly reduce the global disease burden by helping to control morbidity and mortality brought about by various diseases.

Table 8.1 Examples of bioengineered microbes, their disease targets, and the reported mechanism of action

S. no.	Bacterial species	Type of cancer	Mechanism of action
1.	*Mycobacterium bovis* BCG	Bladder cancer	Immune stimulation, enhances the pool of pro-inflammatory cytokines (Shintani Y et al. 2007)
2.	*Streptococcus pyogenes* OK-432 (Chikazu et al. 2017)	Bone sarcoma (Mccarthy EF 2006), lymphangiomas, intraoral ranula	Immune sensitization, increase in neutrophils, macrophages, and lymphocytes (Miwa S et al. 2019)
3.	*Clostridium novyi*	Leiomyoma	Mechanism unknown (Roberts et al. 2014; Barbé et al. 2006)
4.	*Salmonella typhimurium*NVP2009	Pancreatic cancers	Blocks angiogenesis as it has an expression plasmid encoding VEGFR2 (Al-Ramadi B.K. et al. 2009; Gajda et al. 2007)

8.2 History of Bioengineered Microbes

The therapeutic application of microbes predates even the experimental establishment of Koch's "germ theory of disease" and is as old as two centuries. It was a peculiar and fine observational prowess of Vautier in 1813 that captured the fact that those persons who had a progressing tumor and developed gas gangrene experienced tumor regression. It was later established that gas gangrene results from *Clostridium perfringens.* This ultimately indicated that some bacteria may naturally promote tumor regression or growth of some bacteria in tumor cells may result in such cells getting killed. In the late nineteenth century, it was also observed that *Streptococcus pyogenes* natural infection also accelerates the killing of tumor cells. This led to the use of Coley's toxins (killed extracts of *S. pyogenes & Serratia* sp.) for inoperable sarcomas showing potential curative effects. The use of BCG strain of *M. bovis* was also employed to cure bladder cancer, and this practice still finds its use in modern times. The success of BCG strain to cure cancer has been attributed to the fact that this strain in particular is able to induce the production antitumor cytokines (IL-2), TNF-α, and interferon-Y. By extension, it may be possible to predict that along with bacterial cytotoxicity and immunomodulation, these microbes may be affecting the survival of cancer cells.

Probiotics, especially the natural, native microorganisms, had been used for promoting disease therapies in many cases since a century ago or so. A well-suited example would be the use of *Lactobacillus* sp., *Bifidobacterium* sp., and *Leuconostoc* sp. for the treatment of CDAD (*Clostridium difficile*-associated diarrhea). It is an encouraging observation that standard treatment for inflammatory bowel disease (IBD) and Crohn's disease is probiotics. These naturally available probiotic organisms can be better engineered so as to enhance their efficacy by putting in resistance mechanisms to host's tissue environment such as stomach acidity, bile, and increased osmolarity in intestinal tissue.

8.3 Areas Where Bioengineered Microbes Are Currently Used for Cancer Therapy

The currently used bacterial cultures for cancer treatment can be viewed as a supplementary treatment to the standard treatment, enabling complete recovery of the patient (Sun et al. 2008).

The major advantage in using the group of anaerobic organisms in cancer therapy is the fact that unlike chemotherapeutic agents that can spread all over the body tissues, these anaerobic microbes can only grow in tumor microenvironment due to the presence of hypoxic conditions in the latter (Forbes NS 2010).

8.4 Immunomodulatory Recombinant Probiotics

While a lot of natural probiotic organisms are used for therapeutic interventions in many diseases, using the advancement of genetic manipulations, molecular biology can increase the efficiency of such probiotics by making them more suitable for disease therapies (Culligan EP et al. 2009). An example could be the use of such recombinant probiotics to deliver immunomodulatory proteins at sites which are otherwise difficult to access because of physiological constraints such as colon tissue, e.g., excessive inflammatory responses to normal intestinal bacteria are a characteristic symptom in Crohn's disease; IL-10 is a cytokine that can regulate mucosal immune system and thus prevent excessive inflammatory response in such patients. However, systemic IL-10 treatment is not very effective and shows significant side effects. To overcome this, a genetically engineered *Lactococcus lactis* (expressing a mature human IL-10 & made thymine dependent to prevent its growth outside body) was made and showed significant improvement in disease reduction, thereby offering a greater hope with extended therapeutic potential against Crohn's disease.

8.5 Advantages and Limitations

Advantages of such therapeutic interventions include the specificity and highly selective effects of such therapy interventions that make using bioengineered microbes in disease therapy less burdensome and convenient to use (Table 8.1). Other advantages include the capacity to use such bioengineered microbes as in situ production strategies for targeted antitumor and recombinant proteins.

Like every technology, this approach also has some limitations that can be addressed in subsequent times to make it a more robust and gradually increase the acceptance of such therapeutic interventions; few of the limitations for consideration are as follows: most of the studies involving such bioengineered microbes are largely based on animal models of cancer, infection, etc. In vivo stability of the constructs as well as the prevention of horizontal transmission of such vectors from bioengineered microbes needs to be ascertained before being used in actual therapy interventions. (Łukasiewicz and Fol 2018)

8.6 Future Directions

Cancer, infectious diseases, and inflammatory disorders are a rising cause for morbidity and mortality due to such pathologies, especially in the light of increasing antimicrobial resistance; it is imperative to look out and establish the reliability along with validity of alternative strategies to combat such disease modalities. Microbial-based therapies can provide an answer to such problems as demonstrated by ongoing research in microbial bioengineering for disease therapy such as immunomodulatory probiotics, targeted drug and vaccine delivery, and targeted killing of tumor cells.

The future course for this field will depend on the successful transition of such therapies from laboratories to the clinic by accumulating extensive data for safety and efficacy of such bioengineered microorganisms.

8.7 Conclusion

Infectious diseases, cancers, and chronic inflammatory conditions are leading causes of morbidity and mortality all over the world and need immediate attention for newer and more reliable and robust therapeutics especially in the era of the emergence of multidrug resistance. The idea of bioengineered microbes for therapeutic intervention is not a new one, and with recent advancement in many areas of biological sciences, such therapies can offer promising results. However, extensive clinical trials need to be validated before such interventions reach the patient. The major issues of biological containment, preventing horizontal transmission to other species, and greater safety for human use need urgent attention to translate such therapies from laboratory into clinics. The attitude of the regulatory authorities also needs to be changed to be more acceptable toward using genetically modified organisms. The general public should also be made more aware about the potential benefits of such bioengineered microbes even if in the capacity of a complimentary treatment options along with standard chemotherapeutics and radiation therapies.

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