Mini Review

Human and animal intestinal commensals and probiotics vs modern challenges of biosafety: problems and prospects

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ABSTRACT

The appearance of an array of data on the study of the intestinal microbiota in Metazoa has significantly expanded our understanding of the role of commensals in the control of a wide range of physiological functions in higher organisms in norm and pathology. In the intestine, where the microbial load significantly exceeds the number of microorganisms of other ecosystems, the components of the intestinal microbiota are a constant source of stimuli that induce activation of the host immune system. The introduction into practice of biomedical research of innovative high-resolution methods, including multi-omics technologies, has brought data that change our understanding of intestinal commensals, including probiotics with GRAS status, widely used in medicine, agriculture and biotechnology. The ability of these bacteria to induce negative processes in the host body that are beneficial for bacterial proliferation and expansion revealed a clear lack of our knowledge about the logic of their life and the mechanisms of interaction with eukaryotic cells. This determines the urgent need for comprehensive research of probiotics and the development of standardization of their safety assessment. Apriori's confidence in the exceptional benefit of the bacteria widely used in medicine, agriculture and biotechnology has determined the seriously omission in our control system today - the lack of standardization of studies for the safety assessment of bacteria with GRAS status . The moment has come when it became clear that this gap should be promptly filled and that only exact understanding the molecular base of interacting the microbes with eukaryotic cells can provide the foundation for effective practical developments in controlling the evolution of bacterial virulence and probiotic safety strategy, as well as the competent use of genetic technologies for monitoring the environment and managing infectious processes, thus avoiding the dramatic consequences of large-scale interventions in the micro and macro worlds.

Keywords: intestinal commensals, probiotics, innovation technologies, probiotic-host crosstalk, biosafety

1. Context

The appearance of an array of data on the study of the intestinal microbiota in Metazoa has significantly expanded our understanding of the role of commensals in the control of a wide range of physiological functions in higher organisms in norm and pathology (1). In the intestine, where the microbial load significantly exceeds the number of microorganisms of other ecosystems, the components of the intestinal microbiota are a constant source of stimuli that induce activation of the host immune system (2). The co-existence of higher eukaryotes with myriads of microbes is the result of the work of mechanisms that ensure the equilibrium of the microbiome in the holobiont (3). These mechanisms are associated with relative tolerance to residents, but the ability to destroy pathogenic aliens relatively effectively (4). They do not cancel the immunoreactivity to microbial antigens of residents, but cause the hyporeactivity of the immune response (5). The revealed ability of intestinal commensals, including probiotics, to induce negative processes in the host that are beneficial for bacterial proliferation and expansion (6), has become an important aspect indicating the need to correct our ideas about symbionts and probiotic bacteria with GRAS status, as well as their practical applications. The mechanisms underlying these processes are not entirely clear. A significant breakthrough in their research attempts is associated with new research methods (7).

2. Data acquisition

The emergence of high-resolution physico-chemical methods, the introduction of omics technologies into the practice of biomedical research have identified new opportunities for studying the mechanisms of bacterial survival *in vitro*, *in cellula* and *in vivo* under the pressure of biotic and abiotic stressors, in axenic cultures, microbial communities and holobionts (8). Innovative methodological platforms contributed to obtaining unique data relevant to both fundamental and applied science. Experimental results indicating a phenomenally high level of genomic plasticity of microorganisms and the potential for the evolution of bacterial virulence under selective pressure have made significant adjustments to our ideas about the arsenal of self-defense tools in bacteria and prioritization of research (9). At the same time, the growing pool of factual material dictates today the need to focus attention not only on pathogens, but on widespread commensal bacteria with the status of probiotics actively used in medicine, agriculture and the food industry. Reports on the possibility of large-scale genomic reorganization and progressive evolution of virulence in these bacteria under stressful conditions, modulation of host cell signaling systems and suppression of innate immunity, negative regulation of the p53 pathway and induction of DNA damage, disruption of the structure of the intestinal microbiota and cell proliferation (10) revealed a paucity of our knowledge about the "logic of life" of symbionts and the mechanisms of their interactions with eukaryotic cells, which compromises the great ideas and horizons of a number of practical applications (11). All this determines the relevance of comprehensive studies of commensals, their plasticity potential under different environmental conditions, methods of intercellular communication and interaction with regulatory networks of higher organisms, as well as the development of standardization of probiotic safety assessment. Vesicles produced by bacterial cells loaded with bioactive molecules capable of rapidly reprogramming target cells turned out to be important participants in the crosstalk between bacteria and the host organism (12). These nanostructures have opened up new horizons of fundamental research and practical applications, but their implementation will require to fill gaps in our knowledge on a number of issues, as well as solving the problem of the safety of probiotics and the vesicles produced by them (13). A significant gap is also the lack of systematic and in-depth information about the contribution of the commensal bacteria cells, as well as their vesicles, to the epigenetic and metabolic changes in host that occur at the level of individual cells, causing the modulation of immunosensing and the development of local or systemic immunotolerance (14). Considering that MAMPs (Microbe-associated molecular patterns) there are in both symbiotic and pathogenic microbes, the machinery of differential recognition of commensals and pathogens is not clear. It is obvious that in addition to the classical MAMPs, there are other commensal-produced factors (including in the composition of vesicles), which are crucial for the modulation of the innate immunity, bacterial proliferation and expansion. Some facts in favor of this have recently been obtained (15). It is assumed, that bacterial small ncRNAs and some metabolites, in particular amino acids, can be important mediators of reprogramming host cells (16). In turn, in eukaryotic cells, in addition to the canonical PRRs (Pathogen Recognition Receptors), other sensor variants are found that mediate the remodeling host cell

metabolism and the outcome of interaction with bacteria. GCN2-GCN1 proteins have turned out to be important elements of such systems, which regulate the rearrangement of the molecular machinery of the eukaryotic cell in response to stress signals (including infections, as well as ncRNA and metabolites of intestinal commensals), and control the fate of cells - the transition to apoptosis, autophagy, proliferation (17). At the same time, the data obtained recently indicate that GCN2 stress kinase is not only in close relationship with many other stress proteins, but can be a negative regulator of key controllers of antioxidant protection, cell cycle and proliferation, and contribute to oncogenesis (18). The details of the molecular machinery of these processes have yet to be clarified. It is obvious that the elucidation of the role of intestinal commensals in the modulation and tuning of GCN2-associated regulatory networks critical for host physiology should be among the priority areas of biomedical research. The interaction of L-forms of bacteria with eukaryotic cells is a new and as yet unexplored area (Fig. 1) (19). The phenomenon of transformation of intestinal bacteria into L-forms is associated with a serious and still unresolved problem of recurrent infections in humans (20). Taking into account the problem of selective pressure of abiotic and biotic stressors in relation to living systems, including the pressure of antimicrobial drugs (21), it is hoped that the biology of L-forms of intestinal bacteria, the structure and function of the vesicles produced by them, the features of their inherent signatures - MAMPs, as well as their detectors - PRRs and, accordingly, activated signaling cascades, as well as the biology of commensal bacteria in the case of plankton cultures and biofilms, they will also be the subject of future research (22). These new directions, capable of enriching the scientific community with discoveries of "alternative reality" in microbes, seem extremely relevant both for fundamental studies of the "logic of life" of symbiotic bacteria under different environmental conditions, and for the safety of practical applications associated with the widespread use of probiotics.

3. Results

It is clear that the establishment of symbiotic interactions is a complex process of interaction between the signaling systems of both the microbe and the host, and the number of newly discovered signaling systems and their components is growing rapidly, continuously increasing the layers of complexity of cellular processes and complicating the possibilities of their

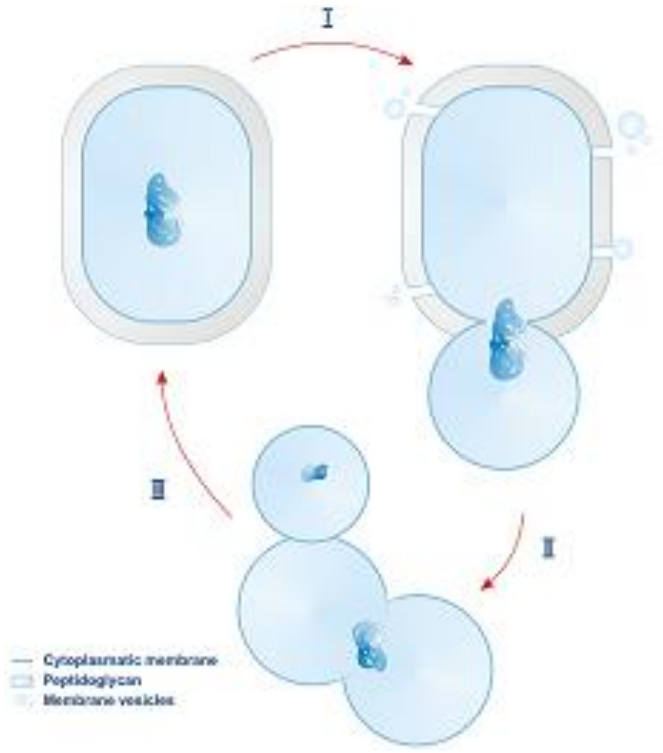

Fig 1. L-form conversion. Emergence of bacterial L-forms may occur in presence of cell wall-active antibiotics, phage and endolysin treatment. Lesions in the bacterial cell wall, disintegration of cell wall due to lytic enzymes induce the transition and may result in releasing bacterial membrane vesicles, which are highly enriched for mRNAs, including SOS-response (I). The wall-less state not only provides bacteria with resistance to respective antimicrobials but retains them ability to proliferate (II). In absence of selective pressure they may revert to walled state (III).

comprehension (23). However, an understanding the molecular mechanisms underlying immunotolerance in relation to intestinal commensals dictates the need for transition from the reductionism of fragmentary non-standardized studies to a holistic representation of both positive and negative aspects of the interactions of the corresponding bacteria with the host on the basis of comprehensive standardized fundamental research based on transdisciplinarity using both classical methods of analysis and modern physico-chemical technologies. Without understanding these mechanisms, the probiotic segment risks not only remaining in the marginal zone, but also leading to dangerous events. It is obvious that modern variants of high-resolution methods, including omics technologies (among them "single-cell" variants), can be of great benefit for the development of this direction, but only on condition of (i) their complex application, (ii) mandatory validation of the obtained data (taking into account the problem of reproducibility of a number of

omics), (iii) integrative analysis of findings and, of course, (iv) standardization of the studies (24).

4. Conclusions

In modern studies of "Intestinal commensals and probiotics vs modern challenges of biosafety", three main directions are distinguished: (i) molecular machinery of the dialogue of the bacteria with eukaryotic signaling systems in model associations; (ii) molecular mechanisms of adaptation to antimicrobial drugs and the evolution of virulence in the bacteria; (iii) structural and functional characteristics of extracellular vesicles relevant bacteria and assessment of their safety.These areas are interconnected and interdependent, which determines the expediency of conducting comprehensive studies to understand the complex effects of intestinal commensals and probiotics on the human and animal bodies. The analysis of eukaryotic cell responses to bacteria and/or their vesicles involves both targeted control of the expression of critical genes and global profiling of RNA, including small RNAs (with subsequent verification and validation of data using targeted control of protein expression and metabolomic profile). Meanwhile, profiling of small pro- and eukaryotic RNAs in multicomponent model systems is by no means a trivial task. Recently, an innovative method for analyzing small RNAs of pro- and eukaryotes in multicomponent systems was presented, associated with the use of the sMETASeq method (25). This method makes it possible to immediately determine the profiles of small RNAs of pro- and eukaryotes in one sample, as well as (based on small RNA sequences) the structure of the microbiota. Unlike DNA-based methods, it allows: (1) to obtain information about the composition of living active organisms, as well as transcription processes occurring in their cells; (2) to investigate the relationship between the small RNAs of the host organism and the microbial composition, as well as between the structure of the microbial community and the pool of small RNAs of microbes and the host; finally, (3) to determine the body's responses to specific stimuli, microorganisms or their components. In this regard, the use of this method in combination with other high-resolution methods to solve the above problems can not only contribute to significant progress in acquiring relevant knowledge, but also to the emergence of breakthrough solutions and the development of new scientific directions.

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Authors' Contribution

Study concept and design: C. V. M., C. O. A., T. M. V. Acquisition of data: C. V. M., C. O. A., T. M. V.Analysis and interpretation of data: C. V. M., C. O. A., T. M. V.

Drafting of the manuscript: C. V. M., C. O. A., T. M. V.

Critical revision of the manuscript: C. V. M., C. O. A., T. M. V.

Ethics

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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