Understanding the mode of action of cosmetics or drugs on skin and its microbiota with omics techniques.

Skin is now composed of a human part and a microbial part which is the more variable part. Up to now a lot of information could be obtained at the diversity, richness and taxonomic levels of microbiotas. Clinical trials are able to show effects which are documented by these descriptive levels but few explanation of effects are clearly described.
Either way, it becomes essential to understand the mechanisms of action and the interaction of the human part with the microbial part to be able to master the safety and efficacy on the skin of drugs and cosmetics.
This is possible with omics techniques, which however have different capacities and performances to answer these questions.
Furthermore, it is now clear with the science of the microbiome that interactions such as the skin-gut axis are of paramount importance, which means that any knowledge of the microbiota can be beneficial for the skin microbiota understanding.

Technical landscape
PCR was discovered around 1985 and allowed to detect specifically DNA strands. Since the years 1990, DNA sequencing techniques opened the door to untargeted approaches of genes. Now NGS allows sequencing a genome at low cost.
Since 1945, mass spectrometry allowed detection of small chemical molecules. For 15 years now, mass spectrometry evolution allows to analyze and quantify proteins. The proteogenomics approach through the genes knowledge and with LC-MS/MS and databases evolutions allows identifying and quantifying proteins in an untargeted mode. Improvements in the mass accuracy, resolution, and sensitivity of MS instruments are enabling the rapid and reliable detection, identification, and quantification of proteins in complex mixtures.
These techniques are opening the door to improved methods for discovering disease-specific biomarkers with the potential to support early disease detection and even individualized therapies.

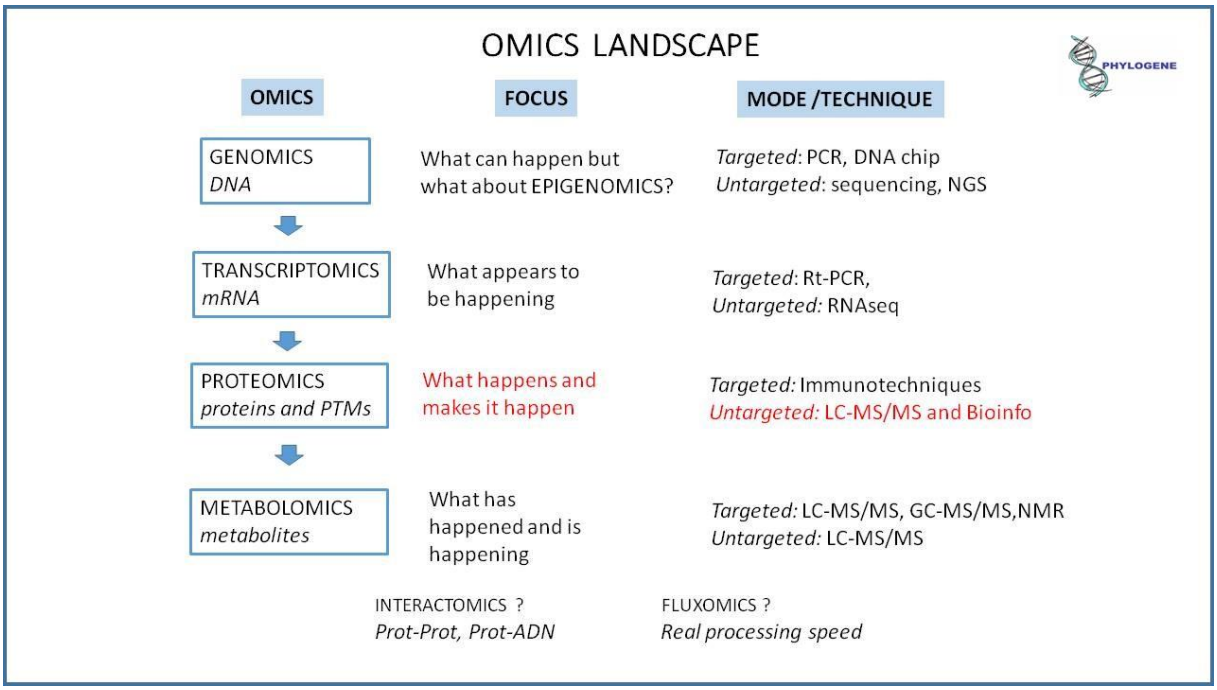

Fig1: Techniques are operational at different steps of the systems biology and provide different levels of information which are targeted specifically on analytes or only assigns a signal to an analyte through databases.

In an untargeted way (shotgun NGS) and quantitatively, a gene gives the information that the translated protein can structure or work in the cell, but we now know that epigenetics mechanisms may interfere or inactivate gene. Genomics stays reasonably at the informational level. (fig.1)
The transcript (mRNA), RNAseq at the quantitative shotgun level, tells us a protein may be translated but if and when remains an open question.
Only LC-MS/MS proteomics tells us which protein is there.
Of course, no technique is able in an untargeted way to tell us if the protein is active and at what speed it produces metabolites, even if the post-translational modifications (PTMs) can be now reached by mass spectrometry (1). Also, no untargeted technique is able to know which protein interact with which other molecule.
Metabolites are produced by proteins and may be metabolized by other proteins. Metabolites may be tracked using LC for the liquid part, GC for the volatile part coupled with MS/MS or NMR but it works rather in a targeted way as databases are poor up to now (2, 3) : of course, there is no structural correspondence between the gene and the metabolite as there is between the gene and the protein.
Shotgun sequencing allows finding all the known genes in a sample, genes which account for around 2% of DNA, the remaining being the “junk DNA” which is now known to be of primary importance. Based on genes sequences databases, LC-MS/MS proteomics can easily identify the functional proteome and may also reach the signaling proteins using sample pre-fractionation. Proteomics has also the “dark proteome” which accounts for around half of LC-MS/MS spectra and corresponds to disordered protein regions but which seems to be essential for the proteome activity disordered regions.

Up to now, metabolomics remains limited in an untargeted mode as only 10% of spectra obtained in mass spectrometry can be identified (2).

LC-MS/MS untargeted quantitative metaproteomics to see human and microbial parts together

Proteomics means the study of expressed **proteins** in a cell, a tissue, an organ or an organism at definite time and conditions. The **proteome** is the entire set of these proteins. Metaproteomics refers to all the proteins of the ecosystem, mainly the host and its microbiotas, named holobiont.
An **LC-MS/MS** instrument analyses continuously the peptide fractions obtained from **HPLC** after enzymatic digestion of a protein extract. The mass spectrometer does every second an MS spectrum followed by further MS/MS fractionation on the most intense components. This information generated on the different proteins segments is compared to mass maps and spectra available in **databases** and it allows the identification of proteins present in samples. This identification is made by using proteogenomics (4) approach which makes the bridge between genes and proteins. The peptide pikes area ratios gives a relative quantification.
Relative quantitative proteomics or metaproteomics refers to the analysis of two or more groups of samples by globally and quantitatively comparing their proteomes. Mass spectrometry has the unique ability to measure changes in complex protein mixtures.
As the identification is a process without targeting particular analytes, this is a hypothesis-free approach.
The analytical workflows are now simple and linear, in a first intent without need of 1D nor 2D prefractionation to reach 5000 proteins in a sample. One challenging difference of metaproteomics compared to proteomics remains the size of the identification databases. The human side contains 23000 genes, but on the microbial side at least 1000 genes per microbial species are filled out.

Associated bioinformatics

The current proteomics LC-MS/MS output data reach easily more than 5000 identified proteins. More than 10000 proteins can be reached easily in metaproteomics. The relative quantification of two conditions reveals tens to hundreds of under- or overexpressed proteins.
This requires heavy data processing pipelines (5) which will analyze all impacted proteins in correspondence with the metabolic pathways in which they are involved. It is so possible to understand the biological events induced by the disease or the dysbiosis.
Usually, a taxonomic analysis, a functional analysis by taxon (Homo sapiens, Fungi, Bacteria and Archae) and inter-functions correlations can be produced which give access to links between signaling/metabolic pathways, potential association of functions to particular taxons, potential interspecific relationships between microorganism and between microorganism and human (6). As a synthesis, the mechanism of action can be formulated (7).

Understanding the effects on host and microbiota

Metagenomics studies initiated with the Human Microbiome or MetaHit projects brought huge knowledge about the individual variability of the microbiotas, the importance of the diversity and quantity of the gut microbiome at the taxonomic level, and relationship between a taxonomic state of a microbiota and diseases. Anyway a huge gap remains to understand the working of the holobiont such as the relations between microbiota and host, the impact of a diet, a probiotics, or a disease. The taxon, genus and species impacted by any dysbiosis and the 2 millions of identified genes in the gut microbiome does not tell much on what really occurs.

Already in 2012, the Human Microbiome Project (8) mentioned that the taxon variability was not in relation with the relative stability of functions at the genomic level.
Metagenomic studies have shown that gut microbiotas share a stable set of core functions, in spite of a large inter-individual structural/compositional variability. However, since sequenced genes are not necessarily expressed, metagenomics cannot provide reliable information on which microbial functional traits are actually changing in response to stimuli from host metabolism, immunity, neurobiology, diet, or other environmental factors which induce a substrate change. (7)
But this type of information can be gathered by functional metaproteomics, which displays higher sensitivity to perturbation and may therefore better reflect host-microbiome interactions (9) and mechanisms of action (10). This way, several correlations were identified between human and bacterial proteins (11) as both human and microbiota proteins are followed.

Using this approach on stool samples it is even possible to functionally relate human proteins to bacterial extracellular vesicles and to identify the peptides of interest reflecting taxonomic and/or pathway changes, which enables further biomarker discovery (12).

In Crohn's disease, metaproteomics and dedicated bioinformatics were used to follow a patient in 4.5 years (6) or a cohort of patients after resection surgery for 1 year (13). Almost all the functions observed across all individuals were observed in multiple phyla, these functions are not specific to any one phylum, genus, or species. There is a clear persistence of conserved metabolic functions across time and individuals. Finally, the gut microbiome's metabolism is not driven by a set of discrete linear pathways but a web of interconnected reactions facilitated by a network of enzymes that connect multiple molecules across multiple pathways (13).

This kind of approach has been recognized with skin microbiota as the reasonable way to further elucidate the extensive intricacies of the skin microbiome. (14) (15)
Furthermore, the huge advantages when studying the skin microbiome are the surface and the symmetry. It allows to compare one side where a cosmetic is applied to the other side where a placebo is applied. Also, the surface allows to use swabbing as an efficient sample collection method. The microbiome is terribly personal and remains sensitive to the environment variations. If there is a need to compare a day 0 to a day X, then the effect of the product can first be validated if no change occurs at the placebo side first.

Conclusion

Even if multi omics approaches can reinforce the information, due to the proteogenomics shortcut, metaproteomics and dedicated bioinformatics seems the most rational and pragmatic approach for discovery and understanding at the functional level the working of skin. This knowledge will allow to design safe and efficient products such as prebiotics, probiotics, postbiotics or regular cosmetics for a healthy skin.

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