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Xenobiotics and the human gut microbiome: metatranscriptomics reveal the active players

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Overview summary

The human gut microbiome plays an important role in the metabolism of xenobiotics. In a recent issue of *Cell*, Maurice et al. identify the active members of the gut microbiome and show how gene expression profiles change within the gut microbial community in response to antibiotics and host-targeted xenobiotics.

The numerous and diverse microbes in the gut (the gut microbiota) have long been known to play key roles in breaking down otherwise indigestible polysaccharides and protecting the host against pathogens through enzymatic pathways encoded for in their microbial genes (the microbiome) (Blaut, 2011; Sousa et al., 2008). More recently, the gut microbiome has been shown to play a role in the metabolism of xenobiotics including antibiotics and drugs targeted against host physiology: at least thirty commercially available drugs are metabolized as substrates by bacterial enzymes (Clayton et al., 2009; Sousa et al., 2008). The human gut's microbial community has been characterized in both healthy and disease states through 16S ribosomal RNA gene sequencing, and Bacteroidetes has been identified as the most abundant phylum in most individuals (Clemente et al., 2012; Human Microbiome Project, 2012). However, this technique cannot identify what portion of the gut microbiome is metabolically active, and its response to, and role in, xenobiotic metabolism (Maurice et al., 2013).

To identify the metabolically active members of the human gut microbiota, Maurice et al. subjected fecal samples to staining with propidium iodide (Pi), DiBAC, and SybrGreen to identify cells with a loss of membrane integrity, membrane polarity, and high or low nucleic acid content, respectively, and then sorted the cells with FACS. Cells with high nucleic acid content (HNA), representing metabolically active microbes, comprised 56.2% of the total (the remaining low nucleic acid content (LNA) fraction was assumed to be inactive). Nearly a third of the gut microbiota had damage through the loss of membrane integrity or membrane polarity. The identity of the microbes in these fractions was assessed through 16S

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rRNA gene sequencing: the HNA fraction was significantly enriched for Firmicutes, specifically Clostridiales, and depleted for Bacteroidales, and the LNA fraction was enriched in Bifidobacteriales. To confirm that the gut harbors a distinct subset of metabolically active Clostridiales, fecal samples from the same individual at two different time points were analyzed using metatranscriptomics (RNA-seq). Significantly higher expression was seen from the Firmicutes phylum relative to the Bacteroidetes, and 15 taxonomic groups from the Firmicutes exhibited expression levels more than 4-fold higher than their gene abundances would have predicted. The metatranscriptomic analysis showed that the active subset of the gut is enriched for Firmicutes, rather than the physically abundant Bacteroidetes phylum.

Consequently, understanding which members of the gut microbiota are metabolically active rather than simply numerically dominant may be critical for predicting individualized responses to drugs. The gut microbiota's response to antibiotics targeted against the microbial community was directly compared to drugs targeted against the host. Exposure to a panel of 6 different host-targeted xenobiotics did not significantly alter microbial community structure. Subjecting the fecal samples to a panel of 8 different antibiotics, however, greatly affected microbial community structure, increasing the percentage of damaged cells in the gut by more than 10%, although the metabolically active HNA subset of Clostridiales was unaffected. Different taxa responded differently to different antibiotics, as expected from previous studies (Dethlefsen and Relman, 2011): for example, *Faecalibacterium prausnitzii* increased 4.5 fold following ampicillin treatment, but decreased under pressure by other antibiotics like ciprofloxacin and tetracycline (Maurice et al., 2013).

What was not expected, however, was the radically different transcriptional responses in the surviving members of the communities to host-targeted drugs vs. antibiotics. Changes in community-wide gene expression were assessed through RNA-seq following perturbation by xenobiotics. Just as microbial community structure is most similar when comparing the same individual at close time points, the transcriptional profiles also clustered by individual rather than by timepoint or treatment (Caporaso et al., 2011; Maurice et al., 2013), underscoring the individualized nature of the microbiome and its transcriptional activity. Nevertheless, across individuals, host-targeted drugs significantly increased expression of genes involved in xenobiotic metabolism and biodegradation, the pentose phosphate pathway, and membrane transport, while antibiotics induced expression of genes involved in phosphate transport, vitamin biosynthesis, translation, and tRNA biosynthesis. Thus, researchers must consider the possibility that the functional profile of microbes that survive antibiotic treatment might be different than their pre-antibiotic selves.

Metatranscriptomics has the ability to detect microbial contributions to pharmaceutical interventions that do not directly result in changes in the microbial community structure. Maurice et al. showed that 328 gene clusters were differentially expressed after exposure to host-targeted xenobiotics even though these same drugs did not significantly alter the microbial community structure. Similarly, when humans, together with mice harboring a defined 15-member community, were dosed with commercially available probiotics including strains of Lactobacillus, Streptococcus thermophilus, and Bifidobacterium animalis subsp.lactis, no appreciable changes were seen in community structure assessed through 16S rRNA sequencing, yet metatranscriptomics revealed significant changes in expression of genes associated with carbohydrate and plant polysaccharide metabolism (McNulty et al., 2011). Gene expression, as well as which species are present and active, is thus likely to be critical for understanding a wide range of microbial processes in the gut.

These changes in gene expression are also important for understanding the unintended interactions between host-targeted drugs and the gut microbiome that can lead to toxic affects. For example, acetaminophen sulfonation is significantly affected by the gut

microbiota: microbial members that produce high levels of p-cresol can greatly reduce the host's ability to sulfonate acetaminophen through competitive inhibition (Clayton et al., 2009). These high levels of endogenously produced p-cresol by gut microbiota might result in increased liver toxicity to the host in response to acetaminophen, and the abundance of p-cresol producing bacteria varies among individuals (Clayton et al., 2009). Thus, understanding which members of the gut microbiota have the capacity to metabolize host-targeted xenobiotics is vital for understanding the drug's effectiveness on an individual basis, and for predicting deleterious side effects. Knowing that significant changes in the microbial community's gene expression can occur in the absence of significant changes in microbial community membership and structure encourages researchers using 16S rRNA sequencing, while very informative in certain situations, to also consider metatranscriptomic profiling (McNulty et al., 2011). Finding microbial signatures at the individual taxa level, community structure level, or gene expression level that correlate with drug efficacy or drug toxicity will be crucial in order for researchers to determine the mechanisms behind drug-microbe-host interactions.

Modern pharmaceutical treatments have a wide range of efficacy levels, and the individuality of human gut microbiomes may play a significant role (Haiser and Turnbaugh, 2012). The use of metatranscriptomics in these types of studies promises to provide insights into the mechanistic interplay between host-targeted drugs and the microbial side of ourselves, and understanding these interactions may assist in development of potent compounds to treat human disease.

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