Theme: The gut and beyond

STUDYING ORGANIZATION AND COMPOSITION OF MUCOSAL BACTERIA BY FLUORESCENT *IN SITU* HYBRIDIZATION (FISH) AND METAGENOME SEQUENCING

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Introduction & objectives: Mucosal bacteria are in close contact with the epithelium where they may contribute to colon carcinogenesis. Studying mucosal bacteria in human biopsies may therefore contribute to a better understanding of microbiota and their role in cancer development. In this study, protocols for fluorescent *in situ* hybridization (FISH) and DNA isolation for metagenomics were evaluated and optimized to study mucosal bacteria in human colon biopsies.

Material and methods: To visualize bacteria in human colon biopsies, a validated FISH protocol was used to stain bacteria with a fluorescent labeled 16s rRNA probe (eub338). FISH experiments were performed on pure bacterial cultures embedded in agar for validation of the eub338 probe (compared to a nonsense probe). Subsequently FISH was performed on human colon biopsies fixed in formalin and methacarn to identify biofilms and invasive bacteria. Additionally, fresh-frozen colon biopsies from tumor and normal tissue were used for DNA extraction. The stool protocol of the HMP was modified for DNA extraction of mucosal bacteria and compared to a commercially available protocol.

Results & Discussion: The FISH protocol was successfully performed on pure bacterial cultures as well as paraffin embedded tissues both fixed in formalin and methacarn. Bacterial biofilms and bacteria invading the mucosa were found on human tumor specimens.

Additionally, a protocol was developed for bacterial DNA extraction from human biopsies that outperforms available commercial protocols. Our optimized protocol required includes PBS washes of human tissue, complete human tissue digestion, followed by a DNAse step to degrade human DNA. Remaining intact bacteria were isolated with our previously optimized bead-beating protocol. Bacterial DNA was successfully isolated from 4 important phyla in the gut with greater sensitivity than other commercial protocols (Firmicutes, Bacteriodetes, Gammaproteobacteria and Actinobacteria). Our novel protocol results in high-molecular weight DNA that can be used for metagenome sequencing.

Both our FISH and DNA isolation protocol for mucosal bacteria from human colon biopsies are reproducible and can be used to evaluate the importance of mucosal microbes in clinical patients.