Symposium 3: Future perspecitves in AR and CRS

Distinct Microbial Composition of Nasal Polyp in Chronic Rhinosinusitis

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Chronic rhinosinusitis (CRS) affects 16% of the U.S. population and costs up to $65 billion annually.[1] However, the pathophysiology of this inflammatory disease is poorly understood, and is complicated by diverse subtypes resulting from divergent and complex interactions of the host immune system and environmental factors.[2-4] Microbiome dysbiosis has been reported to be associated with CRS, but the role of the human sinonasal microbiome in the complex host-environment interplay is yet unclear.[5]

We have examined the sinus microbiota in patients with CRS, and tried to evaluate characteristics of the microbial composition in the nasal polyps. We also tried to find out differences in the microbial composition according to the disease phenotype, anatomical location of the sample, and the methods of sampling.

Study participants

This is a cross-sectional study and patients had undergone primary endoscopic sinus surgery (ESS) in Seoul National University Hospital. Diagnosis of CRS with nasal polyp (CRSwNP) or CRS without nasal polyp (CRSsNP) was based on the European Position Paper on Rhinosinusitis with Nasal Polys.[6] All patients were referral cases and had been initially managed medically with about 3 weeks of systemic antibiotics along with intranasal corticosteroid. Endoscopic sinus surgery was planned when there was no improvement after medical therapy. For controls, patients with nasal septal deviation who underwent septoplasty without any evidence of CRS were recruited. Systemic steroids, and antibiotics were avoided at least the 4 weeks preceding surgery. Demographic variables including age, sex, smoking history, atopy, asthma, antibiotics usage, intranasal steroid usage were evaluated for all patients. Atopy was assessed by a

skin prick test to common inhalant allergens or multiple allergen simultaneous test (Immunosystems, Mountain View, California). Patients with positive spirometric response with methacholine administration and the presence of history of wheezing, shortness of breath, chest tightness or bronchodilator response were regarded as asthma.[7)]

Sample collection

Samples from 23 adults from 3 groups (8 patients with CRSwNP, 7 patients with CRSsNP, 8 control subjects) were harvested. Among study groups, only pre-operative CT scores were significantly different (p=0.001). Other characteristics such as age, male to female ratio, presence of atopy or asthma, usage of topical intranasal steroid, smoking status, and use of antibiotics were not significantly different among groups.

All samples were collected intra-operatively. Swabs were taken from the middle meatus under endoscopic guidance. After swabbing, uncinectomy were performed for all patients. Polyps were harvested in case of their presence during ethmoidectomy. All samples were collected by a single surgeon. Samples were immediately separated into sterile containers, placed on ice, and then transported to the laboratory for storage at -80°C. Harvested DNA samples from the tissue or swab were analyzed by means of molecular phylogenetic analysis of 16S rDNA pyrosequences.

Alpha diversity and beta diversity

Numbers of OTU varied from 10 to 100, and were compared within each patients according to the sample type (polyp vs. uncinate vs. swab). Significant difference was found only between uncinate tissue and swab from patients with CRSsNP. Species evenness as represented by Shannon index showed a significantly lower diversity in nasal polyps than that in uncinate tissue from patients with CRSwNP.

A PCoA based on weighted Unifrac distances which takes genus level OTU data into account was performed. Swab samples showed substantial variations between patients. However, nasal polyps and uncinate tissues showed characteristic weighted UniFrac distances with relatively clustered values.

Phylogenic analysis of microbial genomes

Permutation-based multivariable ANOVA beta diversity test showed that swab samples and uncinate tissues didn't show significantly different microbial compositions at phylum and genus level, regardless of the disease condition (CRSwNP, CRSsNP, control). Since the beta diversity of swab samples were quite high that means big inter-sample differences, I excluded swab samples for further analysis. In order to

control the possibility of disease condition affecting microbial composition of uncinate tissue, phylogenic comparison of polyp tissue had been compared to the uncinate tissues from patients with CRSwNP.

Phylum and genus level comparison between polyp tissue and uncinate tissue

Microbial composition of polyp tissue was significantly different from that of uncinate tissue ($p<0.05$). The bacterial communities from polyps were discrete compared to uncinate tissues. At the phylum level, *Proteobacteria* (mean RA = 44.3%), and *Cyanobacteria* (23.1%) were significantly more abundant in the polyp tissues, while as in uncinate tissues *Bacteroidetes* (mean RA=46.7%), *Firmicutes* (34.2%), *Fusobacteria* (RA=3.1%), *Actinobacteria* (2.1%) were significantly more abundant.

At the genus level, *Sediminibacterium*, JQ650114, *Labilithrix*, *Caulobacter*, and *Sphingomonas* were significantly more abundant in polyp tissues (RA 26.8%, 22.7%, 11.8%, 10.3%, and 6.5%, respectively) compared to uncinate tissues. *Prevotella*, *Barnesiella*, *Terrisporobacter*, and *Ruminococcus* were significantly more abundant in uncinate tissues compared to polyp tissues.

Species level comparison between polyp tissue and uncinate tissue

A distinct microbial composition of polyp tissues could be easily seen in the species level compared to uncinate tissues ($p<0.05$). EF025264, AY375144, HM113065, *Caulobacter segnis*, *Sphingomonas abaci*, EU124820, and FJ269053 were significantly more abundant in polyp tissues. However, DQ796000, *Provotella corbi*, *Brnesiella intestinihominis*, *Terrisporobactor petrolearius* were more abundant in uncinate tissues compared to polyp tissues.

Conclusion

Polyp tissues showed a distinct microbial composition compared to uncinate tissues and swabs. Uncinate tissues also showed a relatively distinct microbial composition according to disease condition, while swab samples did not. For microbial analysis in CRS, the anatomical location of the tissue harvest and sampling method should be considered. In order to evaluate the role of microbiome in the pathogenesis of nasal polyp, further studies on the microbial compositions according to different endotypes and relationship between microbiota and polyps are necessary.

References

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