THE ASSOCIATION OF FUSOBACTERIA IN CUTANEOUS MICROBIOME WITH MELANOMA PROGRESSION.

Chahrazed Mekadim[a*], Jana Čížková[b], Helena Kupcová Skalníková[b], Jakub Mrázek[a]

[a] *Laboratory of Anaerobic Microbiology, Institute of Animal Physiology and Genetics, CAS, Videnska 1083, 14220 Prague, Czech Republic*
[b] *Laboratory of Applied Proteome Analyses, Institute of Animal Physiology and Genetics, CAS, Rumburská 89, 27721 Liběchov, Czech Republic*

[*]mekadim@iapg.cas.cz

Abstract

Melanoma is a malignant skin tumor responsible for major skin cancer death. Various studies have shown that the skin microbiome plays an important role in human and animal health. However, the role of the skin microbiome in the skin tumor microenvironment has not been well investigated.

Objectives: our hypothesis is that both gut and skin microbiomes might be changed during the progression of melanoma. The aim of this study is to characterize the composition of both gut and skin microbiome at different locations (healthy skin and melanoma tissue) of a MeLiM (Melanoma-bearing Libechov Minipig) pig model at different ages.

Methods: DNA isolated from more than a hundred collected samples from feces and skin of MeLiM pigs were used for PCR-DGGE and NGS (Ion torrent platform) of the 16S rDNA. Microbiome analysis of the obtained sequences was done by QIIME2 pipeline and R. qPCR was performed to quantify Fusobacteria in each sample. Fusobacterium was isolated from selected samples through anaerobic conditions. Then, isolates were identified using sequencing of the complete 16S rRNA gene and *gyrB* (housekeeping gene) a molecular marker for *Fusobacterium*.

Results: both bacterial composition and diversity were significantly different between the healthy skin and melanoma tissue microbiomes. PCR-DGGE analysis and quantitative PCR showed the abundance of *Fusobacterium* genera in melanoma samples compared to healthy skin. In addition, a proportional relationship between the age and number of copies of the 16S rRNA gene of Fusobacteria was observed in melanoma samples of MeLiM animals with melanoma progression. Moreover, Fusobacteria was abundant in fecal microbiome of MeLiM compared to fecal microbiome of healthy animals. Four different Fusobacteria strains were isolated from melanoma tissue of different MeLiM animals and identified as *Fusobacterium necrophorum* subsp. *funduliforme* using Sanger sequencing of complete 16Sr RNA gene and *gyr*B gene.

Conclusion: Results show a strong correlation between melanoma development and abundance of Fusobacteria in both gut and skin microbiomes. These observations indicate the importance of microbiota as essential components of the tumor microenvironment. This study may lead to the prediction of diagnostic biomarkers of melanoma by identifying a specific skin microbiome related to tumor progression.

Keywords: Melanoma, Tumor microenvironment, Skin microbiome, gut-skin axis, *Fusobacterium.*