1. **Bitter Melon’s sweet promise: a novel chemopreventive agent by rejuvenating immune system and reprograming of metabolism in oral cancer**

**Global Use of Natural Products in Cancer Patient Management**

**Background**: Oral squamous cell carcinoma (OSCC) is one of the major cancer related deaths worldwide with a 5-year survival rate around 50%. Despite advancement of therapy made during the past few decades, morbidity and recurrence of the disease remain high. Thus, there is a critical clinical need to identify additional preventive and therapeutic strategies. Treatment of dietary product, bitter melon (*Momordica charantia*) extract (BME), displayed anti-proliferative and pro-apoptotic effects against several cancers including OSCC in preclinical models without side-effects. In this study, we investigated the underlying molecular mechanism of BME in OSCC preclinical model.

**Methodology**: RNA-seq, quantitative real-time PCR, western blot and immunofluorescence analyses were performed. To determine metabolites, GC/MS and ESI/MS were performed. Extracellular acidification and glycolytic rate were measured by Seahorse XF analyzer. Generation of reactive oxygen species (ROS) was measured by FACS. Statistical analysis was performed.

**Results and analysis**: BME treatment prevented tobacco associated carcinogen 4-Nitroquinoline 1-oxide induced oral cancer in immune competent mice. RNA-seq analysis revealed that "immune system process" and “metabolic process” are significantly modulated by BME during prevention. Subsequently, we observed that BME treatment significantly reduced pro-inflammatory genes such as, s100a9, IL23a, IL1β and immune checkpoint gene PDCD1 (PD1). In “metabolic process”, BME inhibited key glycolysis and lipogenesis pathways genes. Treatment with BME in oral cancer cell lines significantly reduced pyruvate and lactate levels, and glycolysis rate. Further, we demonstrated that BME treatment significantly reduced phospholipids and calcium-independent phospholipase A2 activity, resulting in modulation of membrane lipid raft composition, endoplasmic reticulum stress and ROS induced cell death.

**Conclusion**: BME treatment prevents carcinogen induced oral cancer development by modulating immune system and reprogramming of metabolic process in pre-clinical model. Thus, as a chemopreventive agent BME has a potential clinical benefit.

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