Molecular Characterization of Cachexia in a Novel Head and Neck Cancer Mouse Model

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Despite accounting for only ~4% of all cancers in the US, experimental and clinical observations suggest that ~40% of patients with head and neck cancer (HNC) present with *'cachexia'*, a severe comorbidity that associates with skeletal muscle defects and is responsible for decreased muscle function, worsened response to treatment, and poorer outcomes. Despite its clinical relevance, the mechanism(s) responsible for the onset of cachexia in HNC patients are not completely understood, thus leading to limited progress in diagnosis and representing a barrier to the development of treatments. Moreover, impeding the identification of viable therapies to combat HNC cachexia is also the limited availability of small animal models. Here, we characterized molecular and functional features of cachexia in a novel, preclinical model for the study of HNC, namely the mouse bearing the B0092 oral squamous cell carcinoma, transplantable in recipient C57BL/6 mice.

In order to elucidate the pro-cachexiogenic features of the B0092 model, we exposed mature C2C12 myotubes to 50% B0092 conditioned medium (CM) and assessed the effects on myotube diameter. The cultures treated with B0092 CM exhibited significant myotube atrophy as compared to untreated controls (-32%, $p<0.001$), also consistent with increased p-STAT3/STAT3 (+2.6-fold, $p<0.0001$ *vs.* C) and reduced p-AKT/AKT ratios (-70%, $p<0.0001$ *vs.* C), altogether indicative of heightened catabolism and decreased anabolism, respectively. To further understand what circulating factors could be promoting the atrophic response in muscle cells, we performed a multiplex cytokine profiling assay on B0092 CM and found dysregulation of several pro-cachexiogenic circulating factors such as LIF (+167-fold) and VEGF (+11-fold) as compared to untreated or 3T3 fibroblast- treated controls. To investigate the systemic effects of HNC tumors, 10-week-old C57BL/6 male mice were implanted with B0092 cells ($5x10^5$ cells, s.c.) and monitored for up to 36 days. Growth of B0092 tumors was accompanied by skeletal muscle atrophy, loss of *Extensor Digitorum Longus* (EDL) muscle strength (-36%, $p<0.001$ *vs.* C), reduced bone mineral density (BMD), measured by DEXA (-8%, $p<0.01$), and decreased trabecular bone, assessed using microCT (Conn.Dn: -34%, $p<0.05$; Tb.N: -12%, $p<0.01$). The muscle atrophic response was also in line with overexpression of the muscle-specific ubiquitin ligases *Trim63* (+1.96-fold, $p<0.05$ *vs.* C) and *Fbxo32* (+1.97-fold, $p<0.05$ *vs.* C), usually upregulated in cachexia models. To identify the gene signature associated with muscle wasting caused by B0092 tumors, we performed Next Gen RNA-Sequencing of quadriceps muscle. The pathway analysis conducted using the *Ingenuity Pathway Analysis* (IPA) and *integrated Differential Expression and Pathway* (iDEP) bioinformatic tools, revealed extreme dysfunction of mitochondria, as well as upregulation of proteasome- and translation-related pathways.

In conclusion, our data suggests that B0092-bearing mice can be a useful tool to uncover novel molecular pathways and potential therapeutic targets for the treatment of HNC cachexia.