Abstract: 71O

Sulforaphane attenuates cancer-induced muscle wasting in C2C12 myotubes

Wenlan Li, Jennifer Trieu, Ronnie Blazev, Benjamin L. Parker, Kate T. Murphy, Kristy Swiderski, and Gordon S. Lynch,

Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, Victoria, 3010, Australia

Cancer cachexia describes the progressive muscle wasting and weakness in cancer patients, which reduces both the response to treatment and overall quality of life, and accounts for nearly one-third of all cancer-related deaths. There is currently no standard treatment for cachexia. Oxidative stress is one of the main contributing mechanisms to the development and progression of cancer cachexia. Compounds that attenuate oxidative stress could potentially protect against cancer-related muscle loss. Sulforaphane (SFN) is a natural antioxidant abundant in cruciferous vegetables, that activates the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway to reduce oxidative stress. SFN reduces cancer cell proliferation *in vitro* and *in vivo* by mitigating oxidative stress (Kanematsu *et al.*, 2011).

In the absence of cancer, preclinical studies have demonstrated positive effects of SFN directly on skeletal muscle, reducing dexamethasone- and serum-starvation induced muscle wasting *in vitro* (Son *et al.*, 2017; Moon *et al.*, 2020), and attenuating muscle damage in mouse models of muscular dystrophy (Sun *et al.*, 2015). Whether SFN can attenuate muscle wasting in the presence of cancer cells remains to be determined. We hypothesised that SFN could attenuate cancer cell-induced wasting in *in vitro* models of cancer cachexia.

To test the hypothesis, immortalised C2C12 mouse muscle myoblasts were differentiated into myotubes and cultured in the presence or absence of colon-26 (C-26) cancer cells for 48 hours. The co-culture system of C2C12 and C-26 cells served as a model to investigate cancer-induced atrophy *in vitro* with C2C12 cells continuously exposed to cancer cell secretions without the direct contact of the two cell types. The chemotherapeutic agent, 5-fluorouracil (5-FU, 5 µM) or vehicle control (dimethyl sulfoxide, DMSO) were added to the myotubes. SFN (10 µM) or vehicle (DMSO) were added for the final 24-hour period. After a 48-hour incubation, myotubes were collected for end-point analyses.

Co-culture with cancer cells in the absence and presence of 5-FU, reduced myotube width by ~30% ($P < 0.001$) and ~20% ($P < 0.01$), respectively, and this was attenuated by SFN in both conditions ($P < 0.05$). Exposure to C-26 conditioned media reduced myotube width by 15% ($P < 0.001$), which was attenuated by SFN. Western immunoblotting and qRT-PCR confirmed activation of Nrf2 signalling and antioxidant genes in response to SFN. Co-administration of Nrf2 inhibitors (ML-385) or MEK inhibitors (PD184352) revealed that attenuation of atrophy by SFN was blocked by ERK inhibition.

These data support the chemoprotective and antioxidative function of SFN in C2C12 myotubes and highlight therapeutic potential for SFN to attenuate cancer cell-related muscle wasting, either as a standalone treatment or in conjunction with current standard treatments, such as chemotherapy. Further *in vivo* investigation of the potential for SFN to treat cancer cachexia is warranted.

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