**ABSTRACT**

Elevated tissue pressure, or interstitial hypertension, has been documented in many types of animal and human solid tumors, including human head and neck squamous cell carcinoma. (Gutmann, Leunig, et al, 1992)

**BACKGROUND**

1. ISF, containing ~0.7 mg of protein, was obtained from neck tumor tissue documented in many types of animal and human solid tumors, including human head and neck squamous cell carcinoma. (Gutmann, Leunig, et al, 1992)

2. Initially, 0.1 mg of ISF was digested with trypsin. Peptides were fractionated by liquid chromatography (LC-MS/MS). Peptides were identified using manual liquid chromatography (LC-MS/MS).

**METHODS AND MATERIALS**

3. Peptides were further separated by strong cation exchange (SCX) HPLC into 12 to 15 fractions.

4. From an initial protein amount of 0.4 mg, 0.15 mg was recovered after ultrafiltration in March 2008. A 27-gauge hollow fiber catheter with 0.1 micron pores was placed into the tumor. Tumor ISF was drawn by vacuum. The ISF was frozen and stored at -80°C.

5. Removal of tumor interstitial fluid has been proposed as a therapeutic measure, and tested in vivo. (DiResta et al. 2000) These investigators developed an Artificial Lymphatic System (ALS) in which transcapillary ultrafiltration continues unabated in the absence of a functional lymphatic system. (DiResta et al. 2000b) This analysis of tumor ISF provides a first glimpse into the ISF proteome and allows identification of biomarkers. Tumors have been shown to contain excess interstitial fluid. The objectives are: 1) To describe a method of removing ISF from solid tumors in the head and neck region and to allow the analysis of ISF for biomarkers that may predict tumor growth and metastasis. (DiResta et al. 2000a) 2) To perform a proteomic analysis of ISF for identification of potential markers.

**RESULTS**

6. Several proteins that were previously found to be potential biomarkers were identified. (DiResta et al. 2000b) These investigators found that removal of approximately 200 ul from the tumor occurred in approximately 20 minutes. (DiResta et al. 2000b)

7. Estimated false discovery rate was calculated after searching against the reversed database. (DiResta et al. 2000b)

8. Each fraction was analyzed on a LTQ-Orbitrap XL mass spectrometer.

9. ISF proteins (0.1 mg) were digested and separated into 24 fractions.

10. The remaining ISF was digested with trypsin and peptides were further separated by strong cation exchange (SCX) HPLC into 12 to 15 fractions.

11. Conclusions: This analysis of tumor ISF provides a first glimpse into the ISF proteome and allows identification of biomarkers that may predict tumor growth and metastasis.

**CONCLUSIONS**

12. Using database searching constraints of 10 ppm precursor mass tolerance and 2 tryptic termini, 804 peptides were identified at a false discovery rate of 1%. (Gutmann, Leunig, et al, 1992) Note that proteins as large as 250 kDa were recovered. Recovery of proteins this size is not possible with currently available microfluidics devices. Several of the proteins identified have been shown to be unique to this fluid. However, some proteins appear to be unique to this fluid.

**REFERENCES**


