

## Abstract (104)

### Introduction:

The repertoire of molecules secreted by smooth muscle cells (*i.e.*, SMC secretome) is a resource for understanding how autologous cell/biomaterial combination products could potentially contribute to regeneration via paracrine signaling mechanisms, in addition to serving as a tool in determining similarities/differences between sources of SMC. We are developing autologous regenerative products for urologic applications including a Neo-Urinary Conduit (NUC) for bladder cancer patients requiring cystectomy, as an alternative to the use of bowel tissue that has many complications (*e.g.*, GI and metabolic disturbances). Bladder cancer patients are typically treated with neoadjuvant cancer drug therapies and/or radiation prior to bladder removal. In this study, we investigated the secretome of three dimensional SMC cultures (3D SMC<sup>1</sup>) initiated from bladder tissue obtained from patients that had undergone cancer treatment. Comparison was made to 3D SMC initiated from non-bladder tissue (SMC derived from subcutaneous adipose tissue) treated *a priori in vitro* with a chemotherapy drug combination, and to normal bladder SMC (control).

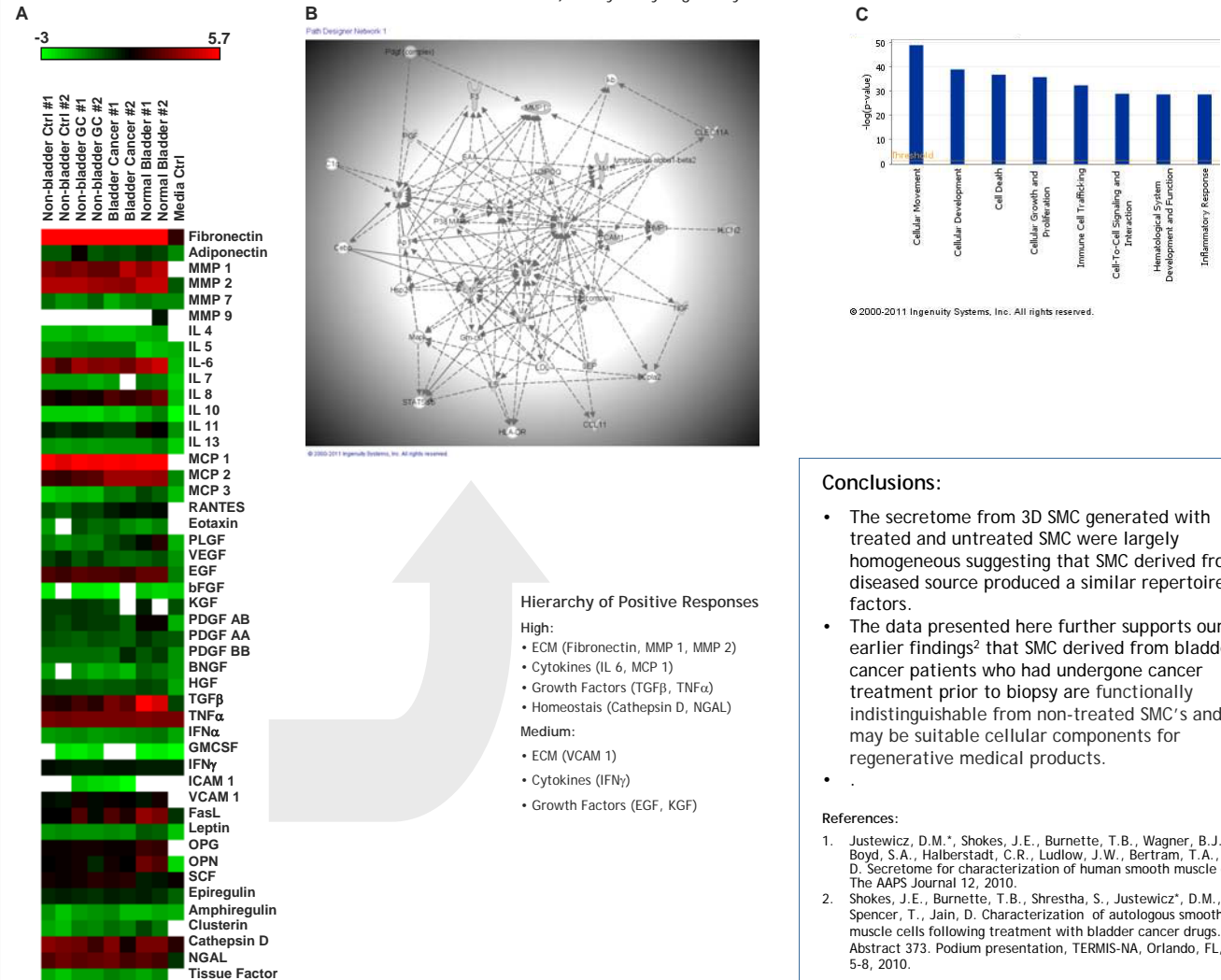
### Materials and Methods:

Bladder SMC were derived from biopsies obtained from bladder cancer patients who had received chemotherapy and/or radiation treatment between three and six months prior to biopsy, and were expanded *ex vivo*. Normal bladder SMC were generated from cadaveric bladders and used as controls. Non-bladder (adipose) SMC were derived from adipose tissue harvests from healthy volunteers. *Ex vivo* expanded SMC cultures were exposed ( $\leq 2$  days) to an inhibitory drug combination comprised of gemcitabine and carboplatin (GC) in an *in vitro* chemotherapy treatment model and allowed to recover in culture after drug exposure. Characterization of the 3D SMC secretome was performed using a scale down model of the NUC clinical product: 1 cm<sup>2</sup> scaffold was seeded with 4x10<sup>5</sup> SMC to produce a construct, and incubated in growth media for six days. Secretory proteins in conditioned media (CM) were examined and analyzed utilizing Aushon Biosystems Searchlight Assay Services and Ingenuity IPA software, respectively.

### Results:

Of the 48 total factors evaluated in the CM of 3D SMC, 39 were present at significant levels (10-2 to 105 pg per 10<sup>3</sup> cells) and were identified as the expanded SMC secretome: Extracellular matrix proteins, pro-inflammatory and chemotactic cytokines, remodeling proteases, growth factors, and apoptotic factors (Fig. 1A). Analysis of the secretome performed with Ingenuity IPA software identified networks that are directly or indirectly related to the ones analyzed in this study (Fig. 1B). In addition, analysis of the secretome with the software determined several biological functions for the factors tested: cellular movement and development, cellular growth and proliferation, hematological system development and function, and cell-to-cell signaling and interaction (Fig. 1C). Overall, comparison of the SMC secretome from bladder cancer patients that had undergone chemotherapy or radiation treatment and *in vitro* GC-treated non-bladder SMC with control bladder SMC indicated that neither treatment resulted in substantial changes in the overall secretome.

Figure 1: A) Heat map of the secretome in CM derived from 3D SMC collected on day 6 of culture expressed in log pg per 10<sup>3</sup> cells. B & C) Analysis by Ingenuity IPA.



### Conclusions:

- The secretome from 3D SMC generated with treated and untreated SMC were largely homogeneous suggesting that SMC derived from a diseased source produced a similar repertoire of factors.
- The data presented here further supports our earlier findings<sup>2</sup> that SMC derived from bladder cancer patients who had undergone cancer treatment prior to biopsy are functionally indistinguishable from non-treated SMC's and may be suitable cellular components for regenerative medical products.

### References:

- Justewicz, D.M.\*, Shokes, J.E., Burnette, T.B., Wagner, B.J., Boyd, S.A., Halberstadt, C.R., Ludlow, J.W., Bertram, T.A., Jain, D. Secretome for characterization of human smooth muscle cells. The AAPS Journal 12, 2010.
- Shokes, J.E., Burnette, T.B., Shrestha, S., Justewicz\*, D.M., Spencer, T., Jain, D. Characterization of autologous smooth muscle cells following treatment with bladder cancer drugs. Abstract 373. Podium presentation, TERMIS-NA, Orlando, FL, Dec. 5-8, 2010.

\* Corresponding author