Quantitative analysis of TGF-ß/SMAD signaling dynamics in a microfluidic co-culture system.

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BACKGROUND: TGF-ß is a pleiotropic cytokine produced by different cell types, including macrophages, which regulates several processes like cell proliferation, differentiation, migration and death). The primary intracellular mediators of its signalling pathway are the SMAD proteins, which are classified as receptor-regulated SMAD (R-SMADs, SMAD2 and SMAD3), common mediator SMAD (Co-SMAD, SMAD4) and inhibitory SMAD (I-SMAD, SMAD7) (Heldin et al., 1997). Temporal control of pathway activity is essential to developmental process and its dysregulation leads to a variety of pathological conditions, including cancer and fibrotic disease. (Warmflash et al., 2012; Pohlers et al.; 2009). Despite a straightforward and simple signalling cascade, TGF-ß can generate different biological responses depending on the cellular context/microenvironment. Different outcomes can be obtained by controlling signal activation, duration and amplitude *via* intracellular and extracellular mechanisms (Zhike et al., 2011;Xiaohua et al., 2016; ten Dijke and Arthur, 2007). The duration of TGF-ß signalling appears to be cell type specific, although the exact mechanisms underlying such a variation are still poorly understood.

METHODS: We designed a co-culture microfluidic platform capable of integrating signalling between different cell cultures. Teflon micro-channels connect two micro-chambers in which different cell types are seeded. Human ES cells and a reporter keratinocyte cell line (HaCaT) containing a TGF-ß1 inducible $(CAGA)_{12}$ luciferase-reporter construct were integrated in the microfluidic platform; this system allows the concentration of TGF-ß secreted by donor cells in the micro-chamber. Exogenous TGF-ß1 or conditioned media derived from human ES cells containing TGF-ß1 was used to activate the TGF-ß1 pathway. Luminescence signal was detected by Vivo Vision IVIS 100 Series (XENOGEN) equipped with Living Image Software version 3.0. rtPCR analyses of signalling proteins were also performed.

RESULTS: We performed quantitative analyses of the luciferase activity associated with TGF-ß1 activation, by measuring the luminescence signal in real-time. We identified a correlation between different TGF-ß1 concentrations and signalling pathway activation. In addition, activation/deactivation kinetics was analysed and we demonstrated that it correlates with the expression of specific activating/inhibitory SMADs. Moreover, a specific TGF-ß1 receptor inhibitor was able to switch off the signalling. The TGF-ß1 response was transient, rising up until 3-5 hrs (peak signal intensity) and returning to baseline within 6-8 hrs. Multiple stimulations affected signalling duration, suggesting a correlation between stimulation frequency and signalling decay. Comparable results were obtained using either exogenously added TGF-ß1 or TGF-ß1 secreted by human ES cells.

CONCLUSIONS: We demonstrated the possibility to study and manipulate diffusible signals by controlling the media transfer in a microfluidic cell co-culture system by carrying out a quantitative and time-dependent real time analysis of of TGF-ß/SMAD signaling. Our microfluidic system allowed us to identify a correlation between dynamic TGF-ß stimulation and SMAD transcriptional response. This work represents a proof of concept that paracrine signals can be successfully modulated in the setting of microfluidic co-cultures.

REFERENCES:

1. Heldin et al. Nature 1997; 390:465–471
2. Pohlers et al. Biochem Biophys Acta 2009; 1792:746-56
3. Warmflash et al. PNAS 2012; 109(28): 1947-56
4. Zhike Z et al., Mol Syst Biol 2011; 7:492
5. Xiaohua Yan et al. J Biol Chem 2016; 291(1):382-92
6. Ten Dijke and Arthur Nat Mol Cell Biol 2007; 8:857-869