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Development of a high-throughput, cell-based assay for anti-myofibroblast activity in Peyronie's disease

Introduction: Peyronie's disease (PD) is a fibrotic disorder characterised by the formation of localised fibrous plaques in the tunica albuginea (TA) of the penis, which can result in pain during erection, deformities and erectile dysfunction. PD has been affecting a growing number of men worldwide, however medical treatment for PD is currently lacking (1,2) and no method is available to quickly and cheaply test a large number of potential compounds, known as high throughput screening (HTS). The aim of this study was to develop and validate an HTS assay for the identification of compounds with anti-myofibroblast activity in cells established from TA.

Methods: Fibroblasts cultures established from the TA of patients with and without PD were exposed to TGF-β1 in order to differentiate them into myofibroblasts. Quantification of alpha-smooth muscle actin (α-SMA) immunostaining was performed using immunocytochemistry (ICC), immunohistochemistry (IHC) and real-time RT-PCR (RT-qPCR). In Cell Western (ICW) method was used to develop the HTS assay measuring α-SMA staining and cell numbers, in cells isolated from non-PD TA tissue and exposed to control conditions, TGF-β1 and 22 FDA approved drugs with potential anti-myofibroblast activity.

Results: PD plaque tissue showed a significantly higher number of α-SMA-positive cells (approximately 3-fold) than non-PD TA tissue. The number of α-SMA-positive cells significantly increased in the presence of TGF-β1 (12-fold) in cells isolated from non-PD TA tissue. The effect of TGF-β1 on cell numbers was mirrored by α-SMA mRNA expression (15-fold). The ICW method was able to reproducibly and effectively detect TGF-β1-induced myofibroblast differentiation, obtaining an average Z' of 0.84 with a CV of 6% in 96 well plates. Using this method, 5 of the 22 drugs were found to significantly inhibit TGF-β1-induced myofibroblast differentiation. For each compound a full concentration response curve (CRC) was constructed, where an inverted sigmoid curve with an upper and lower plateau was observed. The IC_{50} values were calculated for each compound, obtaining an IC_{50} of13.51 µg/ml, 15.81 µM, 7.41 µM, 0.03 µM and 0.30 ng/ml for the drug A, B, C, D and E, respectively. DNA staining showed that the cell numbers were not significantly reduced by any of the five compounds.

Conclusions: A novel assay amenable to HTS was developed for the detection of compounds with anti-myofibroblast activity in cells isolated from human non-PD tissue. Five FDA approved drugs were shown to impede the TGF-β1-induced myofibroblast transformation. The data presented herein suggests that this novel assay can potentially be used for identification of novel compounds or repositioning of approved drugs not only for PD but also for other fibrotic disorders.

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References:

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