

Fibroblasts Transformed to a Wound Healing Phenotype Accumulate a Hyaluronan-Rich Extracellular Matrix Through Reduced Degradation

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INTRODUCTION: Wound healing involves a complex sequence of interactions leading to tissue repair and resolution of injury. Some injuries, however, fail to resolve, resulting in chronic scarring and fibrosis. A common goal of groups investigating scarring and wound healing is understanding the mechanisms differentiating between scarred and scar-free healing. Fibroblasts are central to both the healing and scarring processes and have distinct phenotypes depending on the site from which they are isolated.

The importance of fibroblasts with a myofibroblastic phenotype to the progression of wound healing has been emphasised by the work of several groups in recent years. We have recently investigated differences in glycosaminoglycan (GAG) synthesis, structure and function associated with the possession of a myofibroblastic phenotype compared to that of a normal fibroblast. A major finding was that the important linear polysaccharide, hyaluronan (HA) synthesis was more than 4 fold increased in myofibroblasts. HA is ubiquitously distributed in the extracellular matrix (ECM) and is implicated in the regulation of diverse biological processes including morphogenesis, angiogenesis, tumourigenesis, inflammation, cellular transformation and tissue repair through a direct effect on cell proliferation, phenotype and migration. The present study investigated the synthesis and turnover of HA by these cells.

METHODS: Fibroblasts were differentiated to myofibroblasts by treatment with TGF β 1. HA synthesis was measured by incorporation of ^3H -glucosamine over 24 hrs. HA size was determined following ion exchange and size exclusion chromatography. mRNA levels for hyaluronan synthases (HAS) or hyaluronidases (HYAL) were assessed by RT-PCR. HYAL activity was visualised by HA zymography and degradation of ^3H -HA. HYAL was localized by immunohistochemistry

RESULTS: Upto 4 fold more HA was secreted into the culture medium and pericellular matrix following myofibroblastic differentiation. Inhibition of HAS activity and analysis of mRNA levels, showed that this was not due to induction of HAS enzymes or the increased synthesis of HA. Rather exogenously supplied HA and endogenously synthesised HA were degraded at a reduced rate by myofibroblasts. Immunoblotting of culture supernatants demonstrated more HYAL 1 and HYAL 2 in the myofibroblast medium suggesting it had been shed or secreted. Acidification of the medium to the HYAL pH optimum, however, showed that most of this enzyme was inactive. Immunolocalisation of HYALs and the HA receptor CD44 by confocal microscopy showed a much more diffuse pattern of expression in myofibroblasts, while they were very discretely localised in fibroblasts.

DISCUSSION & CONCLUSIONS:

Both the endogenously increased expression and the over-expression of HA synthase 2 with a resulting increase in HA synthesis have previously been linked to cellular transformation. The results of the current study, however, found no causal link between HA synthesis and myofibroblastic differentiation. Rather there is a major reorganisation of the mechanism for degrading HA which leaves the cell surrounded by an extensive HA-rich matrix.

In vivo this matrix is likely to provide a stabilising environment in which the myofibroblast exists protected from the modifying effects of other cells. To alter this phenotype it may therefore be possible to reverse the changes in HYAL reorganisation. It may be that this would contribute to the generation of a fibroblastic phenotype with the potential for scarless wound healing.