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Characterisation of recombinant hyaluronidase-1 and -3, and of hyaluronan turnover in mineralising osteoblasts

By

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Abstract

The mammalian hyaluronidases (HYALs) represent a family of enzymes that can degrade hyaluronan (HA). This thesis examines the properties of hyaluronidase-1 (HYAL-1) and hyaluronidase-3 (HYAL-3), as well as the production of hyaluronan and the expression of HYAL and hyaluronan synthases (HASs) in mineralising osteoblasts.

Recombinant hyaluronidase-1 (rHYAL-1) has a mass of 57 kDa, of which 10 kDa is due to glycoslyation and 47 kDa is primary protein translation product. rHYAL-1 was shown to not only degrade HA, but also to function as an endo-glucosaminidase in the degradation of the sulphated gags chondroitin sulphate and dermatan sulphate.

Recombinant hyaluronidase-3 (rHYAL-3) has a mass of 46 kDa, of which 9 kDa is due to glycoslyation and 37 kDa is primary protein translation product. Immunofluorescence analysis localised His-tagged rHYAL-3 to the endoplasmic reticulum and lysosomes. *In vitro* activity assays demonstrated that HYAL-3 showed no glycohydrolase activity against any glycosaminoglycan (gag) substrate tested. However, the HYAL-3 knock-out mouse (*hyal-3*$^{-/-}$) accumulates gag in testis, kidney and muscle, suggesting that HYAL-3 has a highly restrictive substrate specificity. A role for HYAL-3 in the testis is supported by previous data that has shown HYAL-3 is highly expressed in human testis.

HA, the primary substrate of HYAL, has previously been implicated to play an important role in the mineralisation of bone. In this study mRNA expression of the HASs that synthesise HA (HAS-1, HAS-2 and HAS-3), and the HYALs which degrade HA (HYAL-1, HYAL-2, HYAL-3, HYAL-4) were examined in an osteoblast cell line

that could be induced to mineralise *in vitro* and gene expression was compared to the amount of gag production. During mineralisation a 13-fold decrease in HAS-3 expression was observed, as well as a 62-fold increase in HYAL-2 expression, a 13-fold increase in HYAL-3 expression and a 3-fold increase in HYAL-4 expression. These changes in gene expression were coupled to a 5-fold decrease in the production of HA. Therefore, in mineralising osteoblasts, expression of the genes that control HA metabolism are co-ordinated such that a general decrease in the expression of HASs and an increase in HYAL expression corresponds to a decrease in HA. These data implicate a role for HA in the early stages of matrix synthesis and maturation, rather than the later process of mineralisation.