112

MODULATION OF HYALURONAN (HA) SYNTHESIS BY HUMAN PERITONEAL MESOTHELIAL CELLS (HPMC) IN RESPONSE TO INJURY.

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HA, a non-sulphated glycosaminoglycan has been implicated to play a pivotal role in cell and tissue injury. We have established an in vitro model of mesothelial injury that will allow us to monitor the synthesis of HA during mechanical injury of the mesothelial monolayer. HA synthesis was analysed by RT-PCR of the hyaluronan synthases (HAS enzymes), incorporation of [^{3}H]-glucosamine into de novo synthesis of HA and localisation of HA by cytochemical staining of uridine diphosphoglucose dehydrogenase (UDPGD) and HABR.

Confluent HPMC were multi-scratch wounded and at selective time periods until the re-establishment of the monolayer, the RNA was extracted and the message for HAS I, II and III investigated. Results showed that HAS I was not present in either control or wounded cells. HAS II was not present in control cells but was found to be induced in the wounded cultures, maximal induction was at 12-24 h after injury. In comparison, HAS III was found to be constitutive in the non-injured cells but decreased once the cells were injured. *De novo* synthesis of HA was found to be up-regulated in the injured monolayers and maximal synthesis was observed 24 h after injury (5.5-fold increase over control), which co-incided with the induction of HAS II. HA synthesis returned to baseline levels after the re-establishment of the wound. No difference was detected in the hydrodynamic size of HA synthesised by non-injured or injured HPMC. Cytochemical staining of UDPGD was identified to the leading edge of the wound and to those cells migrating into the denuded area. This correlated to a similar staining pattern of the cells to de novo synthesis of HA.

In a separate experiment, exogenous toxin-free HA was added to wounded HPMC at concentrations equivalent to those isolated in spent non-infected and infected CAPD fluids and the rate of migration of the cells determined. Results showed that the migration rate of the cells increased in a dose dependent manner. Digestion of the HA to disaccharides showed no increase in rate of cell migration over control, thus suggesting that intact HA is required for cell migration.

In conclusion, HA is an important mediator of cell injury and migration. Although the mechanism to HA synthesis during injury has yet to be fully elucidated, HAS II may in part, play an important role in the up-regulation of HA synthesis.

113

Childhood Absence Epilepsy with Tonic-Clonic Seizures and EEG 3-4 Hz Spike and Polyspike-Slow Wave Complexes mapped to Chromosome 8q24

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A five generation multiplex family from Bombay, India was ascertained through a proband inherited with persistent childhood absence epilepsy (CAE) syndrome. We considered only persons with absence seizures and/or EEG 3-4 Hz spike and multispike-slow wave complexes as affected. Model free affected pedigree member (APM) method was used during initial screening with chromosome 6p, 8q and 1p microsatellites. APM obtained significant p values of 0.00000 to 0.02 for D8S256, D8S537, D8S534, D8S1753, D8S274, D8S1783, D8S502, D8S272, and D8S1761. Two-point linkage analysis, assumed an autosomal dominant mode of inheritance with 50% penetrance, revealed a significant lod score of Zmax = 3.6, at $\theta_{(m=f)} = 0.00$, for D8S502. Subsequent human genome screen revealed no other locus that achieved significant Zmax. We extended the study to five smaller multiplex families, from Spain, Saudi Arabia, Argentina and California, ascertained through probands with the same persisting CAE syndrome. Total pooled lod score was 2.4 for D8S537 at $\theta_{(m=f)} = 0.00$ and 1.7 for D8S1761 at $\theta_{(m=f)} = 0.00$. Haplotypes composed of the same 8q24 microsatellites segregated with affected members of all six families. Recombinations in five affected members of these six families position the CAE gene in a 3.2 cM interval flanked by D8S1710 and D8S502. Further genetic study is desirable at this stage for underlying genetic defect.