Actopaxin: a novel regulator of cell migration and invasion in human hepatocellular carcinoma

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Introduction: The elementary steps leading to the development of metastasis is a complex process involving cell spreading, lamellipodia formation, and cell migration. Actopaxin, a focal adhesion and cytoskeleton-associated protein, is a member of a multi-gene family of which its phosphorylation at Ser/Thr-Pro motifs is required for such processes. The objective of this study was to examine the role of Actopaxin in hepatocellular carcinoma (HCC) cell migration, invasion and development of metastasis.

Methods: The expression of Actopaxin in primary and metastastic liver cancer cell lines was examined by western blot and RT-PCR. The functional effects of enforced expression or down-regulation of Actopaxin was investigated by cell migration, cell invasion, and wound healing assays. The expression of downstream signalling targets of Actpaxin and markers for epithelial mesenchymal transition was studied. Immunofluorescence staining was used to examine the effect of Actopaxin expression on cell shape, stress fibre organisation, and focal adhesion.

Results: The expression of Actopaxin was found to be highly expressed in the metastatic HCC cell lines H2M, MHCC-97L and MHCC-97H as compared with other primary HCC cell lines. Expression of a shorter form of Actopaxin (SF-Actopaxin) was also detected by western blot in some of these cell lines, suggesting the presence of more than one form of Actopaxin in human cells. The SF-Actopaxin lacks a fragment in the C-terminal, resulting in an incomplete second CH domain which consists of binding sites for its downstream activation targets. Enforced expression of long-form Actopaxin (LF-Actopaxin) in PLC, but not its corresponding short form readily enhanced cell migration in wound healing assays. Elevated protein levels of ILK, PINCH and phospho-paxillin, which are involved in focal adhesion complex formation and integrin signalling pathway, were also observed in LF-Actopaxin transfectants. Morphological changes were also observed in PLC LF-Actopaxin transfectants, showing enhanced stress fibre formation, filopodia and lamellipodia (cell protrusions) on the cell surfaces.

Conclusion: This study demonstrated for the first time the pro-migratory effects of Actopaxin in human HCC, and the existence of a short form which lacks a complete CH domain that is critical for cell migration, re-organisation of cytoskeletal events and turnover of focal adhesions. The differential effects of LF- and SF-Actopaxin on ILK, PINCH and phospho-paxillin protein levels might partly explain their influence on cell migration capacity in HCC cells. Further studies are warranted to investigate the potential role of Actopaxin in HCC tumour progression and cell invasioned stress fibre formation, filopodia and lamellipodia (cell protrusions) on the cell surfaces.

Study of arsenic trioxide sensitivity in human leukaemia: role of aquaporin 9 and its transcriptional regulators

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Introduction: Arsenic trioxide (As_2O_3) is an active drug in the treatment of acute promyelocytic leukaemia, and shows much promise in other haematological malignancies. It has previously been demonstrated that the transmembrane protein aquaporin 9 (AQP9) controls cellular arsenic uptake in myeloid and lymphoid leukaemia cells, and thus controls arsenic sensitivity. Consequently, leukaemia cells with higher AQP9 expression are more sensitive to As_2O_3. The objective of this study was to investigate the potential of drug-induced AQP9 upregulation in enhancing the sensitivity to As_2O_3-mediated cytotoxicity.

Methods and Results: Leukaemia cells were treated with the glucocorticoid dexamethasone (Dex) and the demethylating agent 5-aza-2′-deoxycytidine (DAC) separately. Increased AQP9 expression level and enhanced As_2O_3-mediated cytotoxicity after Dex or DAC treatment were demonstrated by quantitative PCR and MTT assay respectively. Treatment with glucocorticoid receptor antagonist RU486 reversed the effects of Dex, suggesting that Dex acted on the transcription of AQP9 through the glucocorticoid receptor. DAC treatment enhanced AQP9 expression in cell lines not expressing AQP9. Since analysis of the promoter region of AQP9 did not reveal any CpG islands, direct demethylation of AQP9 promoter is unlikely and demethylation of other transcription factor genes, which activate AQP9 expression might be involved. Several potential transcription factors were examined. Methylation studies showed that HNF1A was methylated in leukaemia cells. HNF1A expression was detected after DAC treatment and HNF1A siRNA abrogated the DAC-mediated AQP9 level upregulation. These results suggested that DAC increased the expression of AQP9 indirectly via demethylation and activation of HNF1A.

Conclusion: Dexamethasone and 5-aza-2′-deoxycytidine enhance As_2O_3-mediated cytotoxicity through upregulation of AQP9. Synergistic action between these drugs and As_2O_3 has potential therapeutic implication in the treatment of cancers that are resistant to As_2O_3 alone.