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# ELUCIDATION OF THE MOLECULAR MECHANISMS OF ACUTE PHASE PROTEIN GENE EXPRESSION REGULATED BY INTERLEUKIN-6 VIA PEROXISOME PROLIFERATED ACTIVATED RECEPTOR α AND CCAAT/ENHANCER BINDING PROTEINS IN HUMAN LIVER CELLS.

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The acute phase response (APR) is an orchestrated response to tissue injury and infection. A prominent feature of APR is the changes in the expression of liver's proteins, known as acute phase proteins (APPs). It has been reported that IL-6 signalling regulate the activity of transcription factors including PPARα and C/EBPs which in turn, changes the pattern of APP gene expression. However, transcriptional regulation of APP by IL-6 via these transcription factors is not fully understood. Therefore, the aim of this study was to elucidate the molecular mechanisms of PPARα and C/EBP in mediating IL-6-regulated gene expression of APPs. The effects of transcription factors knockdown on the levels of APP mRNA displayed different pattern of changes upon PPARα and C/EBP knockdown with respective small interference RNA (siRNA). Therefore, the results indicated that PPARα and C/EBP members may have different mechanisms of action in modulating the effects of IL-6 on the gene expression of different APPs in HepG2 cells. This might be due to the diverse arrangements of transcription factor binding elements in different APP promoters as predicted using MatInspector. From six APPs, retinol binding protein 4 (RBP4) and transthyretin (TTR) were selected for further characterization of *cis*-acting

regulatory elements in their promoter. It is demonstrated that the region of 500bp between -1,468 to -978 in RBP4 promoter may contain the IL-6 response elements that mediated maximal inhibition of negative APP promoter activity. This region was further analyzed to verify the putative binding sites of PPARα and C/EBP in RBP4 promoter as well as their roles in modulating IL-6 negative regulation of gene expression. Functional studies showed that PPARα and C/EBP binding to their *cis*-acting elements at -1079 to -1057 and -1460 to -1439, respectively, may antagonistically interact to modulate IL-6 regulation of RBP4 promoter activity and gene expression. This interaction is necessary for discrete and precise regulation of RBP4 gene expression in response to external stimuli during physiological changes such as APR. The finding from this study provided additional valuable information to better understand the molecular mechanisms of APP gene expression regulated by IL-6 via PPARα and C/EBPs.

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**ELUSIDASI MEKANISMA MOLEKUL PENGEKSPRESAN GEN PROTEIN FASA AKUT YANG DIKAWAL ATUR OLEH INTERLEUKIN-6 MELALUI RESEPTOR AKTIVASI PEMBIAKAN PEROKSISOM ALFA DAN PROTIN PENGGALAK PELEKATAN CCAAT DI DALAM SEL HATI MANUSIA.**

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Tindak balas fasa akut (APR) adalah respon kepada kecederaan tisu dan jangkitan. Satu ciri menonjol APR adalah perubahan ekspresi protein hati yang dikenali sebagai protein rangsangan akut (APP). Telah dilaporkan pengisyaratan IL-6 mengawal aktiviti factor-faktor transkripsi, seterusnya mengubah corak ekspresi gen APP. Tetapi, pengawalan transkripsi APP oleh IL-6 melalui faktor-faktor transkripsi ini masih belum difahami sepenuhnya. Oleh itu, tujuan kajian ini adalah untuk mengenalpasti mekanisma molekular PPARα dan C/EBP dalam memodulasi pengawalan ekspresi gen APP oleh IL-6. Kesan pengurangan faktor transkripsi ke atas tahap penghasilan mRNA APP menunjukkan corak perubahan yang berbeza apabila PPARα dan setiap jenis C/EBP dikurangkan menggunakan ‘small-interference RNA’(siRNA) tertentu. Oleh itu, dapatan mencadangkan PPARα dan C/EBP mungkin mempunyai mekanisma berbeza dalam memodulasi ekpresi gen setiap APP yang dikawal oleh IL-6 di dalam sel HepG2. Ini mungkin kerana perbezaan susunan elemen-elemen pelekatan faktor transkripsi di dalam promoter setiap APP seperti yang dijangka menggunakan software ‘MatInspector. Daripada enam gen APP, retinol-binding protein 4 (RBP4) dan transthyretin (TTR) telah dipilih untuk pencirian secara lebih lanjut elemen-elemen tindakan di dalam promoter yang bertanggungjawab

memodulasi kesan perencatan IL-6 ke atas pengekspresan gen. Dapatan menunjukkan segmen bersaiz 500bp yang berada diantara - 1,468 ke -978 di dalam promoter RBP4 mempunyai elemen respon IL-6 yang mengantara perencatan maksima promoter APP negatif. Segmen ini dianalisis secara lebih lanjut dan hasil kajian menunjukkan pelekatan PPARα dan C/EBP, masing-masing pada elemen-elemen tindakan di kedudukan -1079 ke -1057 dan -1460 ke-1439, mungkin berinteraksi secara antagonis dalam memodulasi pengawalan aktiviti promoter dan ekspresi gen RBP4 oleh IL-6. Interaksi ini diperlukan untuk pengawalan ekspresi gen RBP4 yang tepat dan spesifik sebagai respon kepada ransangan luar semasa perubahan fisiologi seperti APR. Dapatan daripada kajian ini telah memberikan informasi tambahan yang penting untuk lebih memahami pengawalan ekspresi gen APP oleh IL-6 melalui PPARα dan C/EBP.