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Comprehensive proteomics of retinoblastoma using iTRAQ labeling and mass spectrometry

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Retinoblastoma (RB) is an intraocular cancer found in children and occurs due to the inactivation of both alleles of Retinoblastoma (RB1) gene located at the 13q14 region of chromosome 13. Abnormality/loss of RB1 gene initiates retinoma and genomic instability which primarily leads to retinoblastoma and is also involved in the progression of other cancers. We performed a high throughput comparative proteomic study to identify differentially expressed proteins in retinoblastoma using iTRAQ labeling and Orbitrap mass spectrometry. In the present study we report first comprehensive proteomic signature of Retinoblastoma. Proteomic atlas was obtained by proteomic analysis of Total proteome, Membrane proteome and Phosphoproteome using Retina and retinoblastoma primary tumor. We identified 897 differentially expressed proteins in the total proteome out of which 398 are upregulated and 499 were down regulated. Selective novel proteins from the mass spectrometry were evaluated on individual tumors by immunohistochemistry. CHGA, AHSG, ApoA2 IGF2BP1 and MDK were up regulated in Retinoblastoma. Knock down studies of novel identified protein revealed IGF2BP1 as a potential target molecule in retinoblastoma. We identified 931 differential proteins in membrane proteome out of which 419 were upregulated and 512 were down regulated. Two novel membrane proteins in the context of retinoblastoma LMNB1 and TFRC are validated using immunohistochemistry in primary tumors. Pathway analysis reveals dysregulation of lipid metabolism and photo transduction pathways. In conclusion our study provides comprehensive proteomic atlas of Retinoblastoma.

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Target and untargeted identification of oxylipids in biological samples by tandem mass spectrometry

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Polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA-C20:4), eicosapentaenoic acid (EPA-C20:5) and docosahexaenoic acid (DHA-C22:6) are precursors in the biosynthesis of bioactive oxylipids known as eicosanoids and docosanoids, which are involved in many immunological and physiological processes. The position at which an oxygen is inserted into the PUFA chain is highly controlled by regio- and stereo selective enzymes, such as LOX, COX and cytochrome-P450. In biological systems, they are minor components present as isomeric and isobaric species. In this sense, Mass Spectrometry (MS) have shown great potential for quantification and identification of these lipid species. In this work, we present the versatility of tandem mass spectrometry for target and untargeted analysis of oxylipids. The developed methods were applied to analyze a hypothalamus rat extract. Using the target LC-MS/MS approach, 10 pre-selected eicosanoids were identified in the crude extract. The untargeted LC-MS/MS approach enabled the detection of several mono and poly oxygenated AA products, which were not identified when using the targeted method. Their structures were proposed according to individual product-ion mass spectra achieved via collision induced dissociation. In conjunction, these MS methods represent powerful strategies to accelerate the identification of oxylipids, and can strongly contribute to the discovery of novel lipid mediators in biological samples.

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