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Establishment of a specific Real-time RT-PCR protocol to detect human leptin receptor isoforms

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The hormone leptin plays a major role in obesity, but exhibits additional crucial functions as a cytokine in reproduction, immunity and even cancer. In humans, four different isoforms of leptin receptors (ObRl, 219.1, 219.2, 219.3) have been described, which share an identical extracellular domain, but differ considerably in their intracellular domains indicating that they might have different cellular functions. Among the leptin receptor isoforms, the signal transduction pathways originated from ObRl have been intensively investigated. The activation of STAT3 (signal transducer and activator of transcription) is triggered via binding to the ObRl-exclusive box 2 motive, while the MAPK (mitogen-activated protein kinase) pathway can be activated through the junxtamembrane box 1 motive, which is present in all isoforms. However, the expression and functions of the 219.1, 219.2 and 219.3 isoform are completely unknown.

To obtain a deeper understanding of the functional role of leptin receptor isoforms, we screened a large number of human cell lines originated from different tissues and organs for their leptin receptor isoform expression pattern. Since reliable protocols for the detection of differential gene expression of human ObR isoforms did not exist, we established and optimized a novel reliable real-time RT-PCR protocol using isoform-specific primers and performed detailed quantitative analyses of the ObR isoform expression. Our results show a ubiquitous expression pattern of all isoforms in several cancer cell lines indicating that ObR can function as an important signalling molecule in many tissues. The insights raised from this analysis

might be relevant for understanding the diverse effects of leptin.