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Quantitative real-time polymerase chain reaction of PDGF-R alpha mRNA expression in B16F10 mouse melanoma cell line

PDGF-R alpha mRNA expression was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA (2 μg/sample) extracted from HUVEC cells, murine B16F10 and primary mouse fibroblasts was prepared using TRIzol (Invitrogen) according to the manufacturer's instructions. mRNAs levels were analyzed using the SYBR-GREEN qPCR method Qiagen. qRT-PCR was performed on cDNAs obtained following retrotranscription of mRNAs.
cDNAs were used as templates for Taqman qRT-PCR with ABI Assays-on Demand on an ABI Prism 7000 sequence detection system (Applied Biosystem). Primers for murine and human PDGFR-alpha were designed with the Primer Express software version 2.0. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified on the same plate for each sample for normalization purposes. The experiments were performed in triplicate; data are reported as average and S.D.