

Signal transduction

- 161 Redundancy of Radioresistant Signaling Pathways Originating from Insulin-like Growth Factor I Receptor
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The insulin-like growth factor I receptor (IGF-IR) has the ability to confer clonogenic radioresistance following ionizing irradiation. We attempted to determine the downstream pathways involved in IGF-IR-mediated radioresistance, and used mouse embryo fibroblasts deficient in endogenous IGF-IR (R-) as recipients for a number of mutant IGF-IRs. Mutational analysis combined with specific inhibitors of phosphatidylinositol-3 kinase (PI3-K) or MEK revealed that IGF-IR mediates clonogenic radioresistance through a number of redundant survival signals of differently weighted relevance, including PI3-K, MEK/ERK, and 14-3-3 proteins. Existence of this kind of redundancy in IGF-IR-mediated radioresistance may have implications for clinical strategies based on molecular targeting of tumor cells overexpressing IGF-IR.

- 162 Radioresistant signaling mechanism induced by Insulin-like growth factor I receptor (IGF-IR) in 3Y1 cells lacking Insulin receptor substrate-1 (IRS1)
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We have found that IGF-IR induces clonogenic radioresistance after gamma-irradiation using mouse embryo fibroblasts deficient in IGF-IR. In this event, its downstream signaling pathways such as MAPK, PI3-K and 14-3-3 proteins were redundantly involved in radioresistance as revealed by mutational analysis; radioresistance was completely retained even if one of three pathways is blocked. Here, we examined the redundant mechanism using another cell line, rat 3Y1 fibroblasts lacking IRS-1, which is crucial for stimulating the PI3-K pathway through IGF-IR. Expression of IGF-IR, but not IRS-1 in 3Y1 cells induced clonogenic radioresistance, suggesting that IGF-IR is able to induce radioresistance also in this cell line, in the absence of activation of the PI3-K pathway. Interestingly, mutant IGF-IR in which the tyrosine residue at 950 is mutated to phenylalanine did not induce radioresistance. This site is a constituent of the NPXY motif, which is a binding site for IRS-1 and Shc. The mechanism regarding this phenomenon is currently under way.

- 163 Stress-inducible hSNK gene regulation in thyroid cancer cells
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Question of biological relevance of human homologue of mouse serum-inducible kinase, hSNK, was addressed in this study. hSNK belongs to polo-like kinase family whose members exert their function at several stages of mitosis. hSNK expression was found to be rapidly upregulated after the irradiation of primary human thyrocytes. Characterization of hSNK promoter structure revealed that activation elements including the TATA-like motif and GC box resided within first 122 nucleotides upstream of an analogous transcription start site. Radiation and other stresses induced the increase of hSNK mRNA levels in ARO cells. The upregulation of hSNK expression was dependent on the presence a positive regulatory element located between nucleotides -85 and -65 of the promoter identified as CRE. Mutation of this element significantly decreased hSNK promoter activity and stress response activity of the -122 construct upon the challenge. We further demonstrate that stress-induced transcriptional activation of hSNK in ARO human thyroid cells is dependent on CREB binding.