UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI | Doctorate program Milan EXPERIMENTAL MEDICINE

MOLECULAR MECHANISMS REGULATING INSULIN LIKE GROWTH FACTOR (IGF) SYSTEM IN ADRENOCORTICAL CARCINOMAS

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Adrenocortical carcinoma (ACC) is a rare endocrine tumor deriving from the adrenal cortex, with incidence of 1/million/year, that increases in the 1st and 4th-5th decades of life, and with a higher prevalence in females. Although heterogeneous, the prognosis is generally poor, with median overall survival of 3-4 years, 5-year overall survival less than 50% in most series, and in metastatic disease less than 20%. Complete surgical resection represents the only curative option, but recurrences are frequent (30-85%). The adrenolytic agent mitotane is the only approved drug for treatment of advanced ACC, but its efficacy is limited, and novel treatment are urgently needed.

The most frequent molecular change of ACC is the overexpression of IGF2, and the consequent activation of IGF signaling system promotes cancer cells growth in an autocrine loop. The complex IGF system includes different IGF ligands (IGF1, IGF2, insulin), and different receptors (IGF1R, IGF2R, and two isoforms of insulin receptor (IRA, IRB)). IGF1R and IR are receptor tyrosine kinases (RTK) that differ in ligand affinity and signal transduction. The specific roles of multiple players involved in the final cellular response to IGF2 autocrine stimulation are poorly identified. In particular, a very specific IGF2 receptor is IGF2R, a scavenger receptor that internalizes and directs IGF2 to lysosomes for degradation, but its role in ACC remains to be investigated. IR exists in two splicing isoforms, IRA, lacking exon 11, and IRB, including exon 11. IRA binds insulin or IGF2, that activate different signalling pathways, including mitogenic effects mediated by IGF2 binding. On the contrary, IRB predominantly binds insulin and is involved in the regulation of the metabolic functions. IRA overexpression may contribute to mediate IGF2-induced cell growth, and IR splicing alterations, leading to increased IRA/IRB ratio, may enhance IGF2 mitogenic effects.

This project is aimed to investigate the molecular factors involved in the regulation of the autocrine proliferative loop induced by IGF2 in ACC, with the final aim to identify new strategies to prevent cancer cells growth.

The specific aims will be to test:

- IGF2R expression, functions and regulation in ACC cells;
- the contribution of IR isoforms to IGF2 effects.

We will use human ACC cell lines H295R and MUC-1, and ACC primary cell cultures obtained from surgically removed human tumours.

IGF2R will be silenced, transfected or stimulated with retinoic acid (RA) to test IGF2 endocytosis/degradation. IGF2 signal transduction will be tested measuring cell proliferation, viability and migration. IGF2R intracellular localization, membrane translocation and function will be tested after insulin, IGF2 or RA incubation. The effects of RA on IGF2R ability to internalize IGF2 will be tested by fluorescence labelled IGF2 or by immunofluorescence, lysosomal markers colocalization, and by measuring IGF2 amount.

To test specific roles of the two isoforms of IR, we will use specific siRNAs for IRA and IRB, and we will test IGF2-mediated and insulin-mediated signalling (proliferation, apoptosis, migration, ERK and AKT phosphorylation; anabolic control, glucose homeostasis, glucose consumption, lactate/pyruvate ratio, glucose uptake assay). Moreover, IR splicing will be manipulated by pharmacological strategies (pladienolide-B, SRPK1 inhibitors). The effects of IRA/IRB unbalance downstream on cell growth induced by IGF2 will be tested.

The results of this study are expected to identify key molecular factors regulating IGF system activation in ACC, leading to the identification of alternative strategies to block IGF2 proliferative potential.

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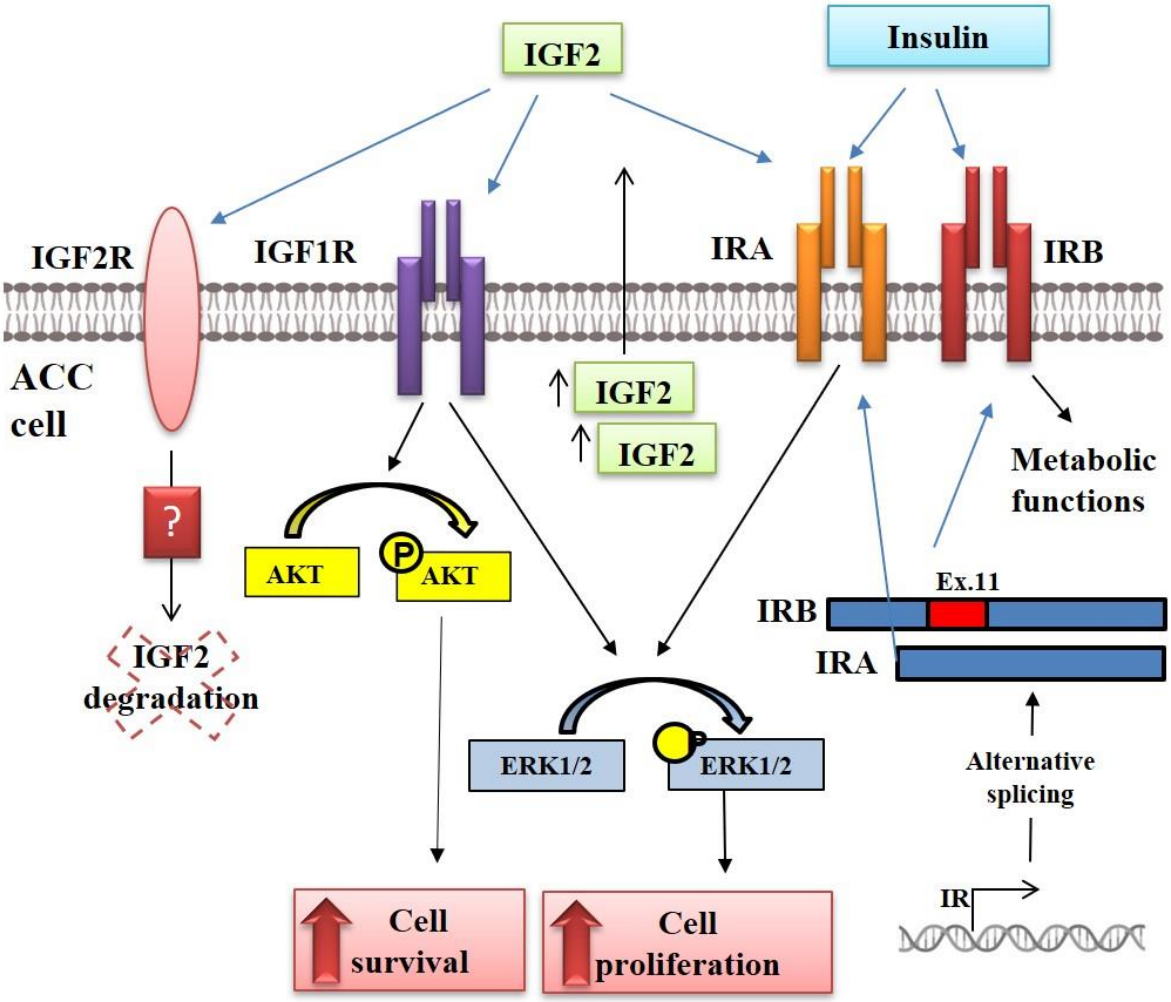

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CANDIDATE SPECIFIC REQUIREMENTS: Cell cultures; cell transfection and silencing; protein detection (western blot, co-/immunoprecipitation); nucleic acid extraction, PCR.

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