Research Interests

1. Signal Transduction and Gene Expression in Reproduction and Cancer

The field of signal transduction deals with attempts to understand the molecular mechanisms by which external stimuli affect target cells. We are interested mainly in G-protein coupled receptors (GPCRs), which are the largest group of membrane receptor, involved in signal transduction with >800 members in humans (~3% of the genome). The GPCRs transmit signals of diverse ligands, such as light, cations, odorants, tastes, hormones and neurotransmitters. The GPCRs are targets for >40% of the currently used therapeutic drugs and they are divided into: class A Rhodopsin related receptors, class B Secretin and Adhesion related receptors, class C Glutamate related receptors and the Taste2, Frizzled and Vomeronasal related receptors. We specialize in the Gonadotropin releasing Hormone (GnRH), which is the first key hormone of the reproductive axis and is a member of family A of GPCRs (1-4 and references therein).

Although critical for reproduction, the mechanism of action of GnRH upon gonadotropin (LH and FSH) release and gene expression is not fully understood (1-3). In addition, chronic administration of GnRH analogs desensitizes the pituitary gonadotropes. Also, GnRH receptors have been found in various sex-hormone dependent tumors', and GnRH analogs exert a direct negative effect on tumors growth (4-6). Thus, GnRH-analogs are presently the main treatment for *in-vitro* fertilization (IVF) and gonadal sex-hormone diseases maily prostate cancer (5-7).

The GnRH receptor has seven transmembrane (TM) domains characteristics of the GPCRs super family. The GnRH receptor is however unique among mammalian GPCRs in lacking a carboxyl terminal tail, which mediates receptor desensitization and internalization in other GPCRs. Consequently the receptor does not undergo rapid desensitization *in-vitro*. We have identified a potential inhibitory site in the GnRH receptor (8).

Following the binding, GnRH receptors initiates a signalling cascade which includes activation of Gqα, PLCβ, Ca^{2+} mobilization and influx selective isozymes of protein kinase C (PKCs) (9), followed by the activation of the mitogen-activated protein kinase (MAPK) cascades (10-12). The MAPK cascades enable transfer of biological information from the receptor to the nucleus for transcriptional regulation. MAPKs can also affect cytoplasmic targets. We investigated the role of the various MAPK (ERK1/2, JNK1/2 and p38) in the differential activation of gonadotropin (LH and FSH) subunit gene transcription (13-15). We are currently studying the integrative signalling network of the GnRHR.

Among others, cell growth is determined by the balance between mitogenic signals that stimulate growth and programmed cell death (apoptosis). The imbalance of growth regulatory mechanisms with an increase in uncontrolled proliferation is a hallmark of the cancer cell. Prostate cancer is the second most frequent tumor in men in the Western world. At initial stages, the growth of prostate cancer is androgen-dependent. When GnRH

analogs are administered in a sustained fashion, GnRH binds to the pituitary gland, desensitizes its normal function and reduces serum testosterone levels, known as “chemical castration” (5-7). Therefore, GnRH synthetic analogs (agonists and antagonists) are now a major treatment for prostate cancer. At later stages the cancer may become hormone-insensitive and at this stage there is no cure. We decided to study further if GnRH analogs may be beneficial also for this later stage. The binding sites and direct actions of GnRH analogs on prostate cancer cells have been described, indicating a direct action of GnRH analogs. Indeed, we have demonstrated direct apoptotic effects of GnRH analogs upon the androgen-insensitive prostate cancer cell line DU-145 (5). Understanding the signal transduction of GnRH in prostate cancer may offer new avenues for therapies.

GnRH is synthesized in the hypothalamus and does not reach the gonads or the prostate. Why are there GnRH receptors in prostate cancer in the first place? Prostate cancer growth is apparently modulated by a paracrine/autocrine network of survival vs. apoptotic signaling molecules involving locally produced GnRH or a GnRH-like material, which might counteract the mitogenic response elicited by local growth factors. Why is it so important to elucidate the mitogenic effects and signaling of GnRH receptors in prostate cancer cells? One of the major treatment options available to men diagnosed with prostate cancer is hormonal therapy. Depending on the stage of the cancer (Gleason scores, etc.), hormonal therapies may be accompanied by other therapies, such as external beam radiation, radioactive seeding, or surgery. However, after 2-3 years the cancer may become hormone-insensitive and at this stage there is no cure. It is obvious that understanding the molecular mechanisms involved in GnRH analogs upon prostate cancer cells proliferation may provide the base of information to develop novel efficient therapies (5-7). In addition if we identify the second messengers involved in this process, additional new targets for growth inhibition ("kill the messenger") will become available. Our study may open a vista for the design of novel combined therapies for prostate cancer. Since there is no cure for androgen-insensitive prostate cancer, it is desired to find GnRH analogs in combination with other signaling molecules able of inducing apoptosis in prostate cancer models in vitro and in vivo. We intend to carry out a systematic study comparing the signaling networks elicited by the GnRH receptors in pituitary vs. androgen-sensitive and insensitive prostate cancer cells (38).

2. Human sperm protein kinases: a novel role in human sperm functions

PKC and MAPK are key regulatory enzymes in cell signaling. We have demonstrated the presence of PKC and MAPK in human sperm (16-21). The bulk of PKC was localized in the equatorial segment of the sperm head. Activation of sperm PKC enhances motility and acrosome reaction. Regression analysis revealed excellent correlation between the percentage of PKC-stained sperm and motility among various donors (16-18). More recently we found that the MAPKs, ERK1/2 and p38 are primarily localized to the tail of mature human spermatozoa (19-21). ERK1/2 stimulation is downstream to PKC activation. ERK1/2 stimulates and p38 inhibits forward and hyperactivated motility, respectively, as yin-yang regulators. Both ERK1/2 and p38MAPK are involved in the acrosome reaction.

Inverse correlation was obtained between the relative expression level of ERK1, and the relative activation level of phospho-p38 and sperm motility, forward progression motility, sperm morphology and viability (20). Therefore, increased expression of ERK1 and activated phospho-p38 can predict poor human sperm quality. Our studies, in addition to shedding light on basic physiological aspects of PKC-MAPK signaling and sperm physiology, might be applied to IVF programs, veterinary medicine and the development of contraceptive vaccines for the 21st century.

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Publications (10 last years)

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