The role of peripheral gonadotropin-releasing hormone receptors in female reproduction

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Gonadotropin-releasing hormone (GnRH)

GnRH

- 10 aa. peptide
- secreted from hypothalamus
- key regulator of reproductive function

GnRH receptor

- on pituitary → release LH and FSH → steroid hormone from gonads
- gene located on Chr 4
- 328 aa. protein, 7-transmembrane-domain G protein-coupled receptor (GPCR)

GnRH receptor

- lacks intracellular carboxyl-terminal domain
- owns very short extracellular amino terminus of only 35 residues
  - → one of the smallest GPCRs
- **expressed not only in the pituitary, but also in normal and tumoral peripheral tissues**
  - physiologic functions
  - possible clinical significances

Outline

literature reviewed, from Pubmed database, published before 2010

- Distribution of peripheral GnRH receptors
- Central versus peripheral GnRH receptor
- Physiological functions and mechanism of actions
- Clinical application

Distribution of peripheral GnRH receptors

- In 1981, the presence of GnRH receptors in human placenta was reported.
  - mRNA in the human placenta and localized to both cytotrophoblast and syncytiotrophoblast cell layers
  - cDNA, full-length isolated from various human placental cells, including choriocarcinoma cell line, immortalized extravillous trophoblasts, and 1st trimester cytotrophoblast cells

Presences of GnRH receptor in ovary ?

- mRNA expressed in multiple ovarian cell types…
  - preovulatory granulosa cells
  - luteinized cells
  - ovarian compartments other than follicular or luteal structures, across different functional stages

Other sites?

- breast
- endometrium (not adenomyosis or myometrium)
- tumor cells
  - Endometrial carcinomas
  - Leiomyomas
  - Leiomyosarcomas
  - Breast cancer
  - Choriocarcinoma
  - Epithelial ovarian cancer
  - Stromal tumors of ovary
- prostate tissue
- liver
- heart
- skeletal muscle
- kidney
- melanoma

GnRH-II / GnRH-II receptor

- **GnRH-II**: decapeptide, 3 aa. different from GnRH
  - recently found in human
  - distributed in the central and peripheral nervous system and peripheral tissue
- **GnRH-II receptor**
  - cloning from fishes, amphibians, and primates → probable existence in humans ?
  - gene on Chr 1 (putative)
  - To date, direct evidence to demonstrate the existence of full-length functional GnRH-II receptor RNA transcript in human tissue is lacking.

Central vs. peripheral GnRH receptor

TABLE 1

Comparison of central and peripheral GnRH receptors.

	Central GnRH receptor	Peripheral GnRH receptor
Distribution	Pituitary	Normal and tumoral reproductive tissues and nonreproductive tissues
Complementary DNA sequence	Same	Same
Affinity for native GnRH	Same	Same
Affinity for agonist buserelin	High	Low
Action of cetrorelix	Competitive antagonist	Agonist
Intracellular activity caused by activation of receptor	Stimulatory—increases gonadotropin synthesis and secretion	Inhibitory—decreases cell proliferation in tumors
Signaling pathway	Gq/11 protein stimulates PLC which	Gi protein activates cAMP and PTP which reduce activity of MAPK
Receptor expression on cell surface	Dynamic, highest level before LH surge	Dynamic in ovary, depends on degree of follicular development and stage of estrous cycle

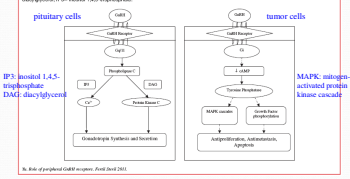
antiproliferative activity on tumor cells

- transcription, translation process
- Whether protein structure are identical ? multpile isoforms ?
- Affinity for agonist: pituitary **100X** higher than placenta
- Mechanism of cetrorelix is unclear → difference at molecular level

Signaling pathway

FIGURE 1

GnRH intracellular signaling pathway in pituitary cells (left) and tumor cells (right). cAMP = cyclic adenosine monophosphate; DAG = diacylglycerol; IP3= inositol 1,4,5-triphosphate.



IP3: inositol 1,4,5-triphosphate
DAG: diacylglycerol

MAPK: mitogen-activated protein kinase cascade

Ix. *Role of peripheral GnRH receptors*. Zeitl Zeitl 2011.

TABLE 1

Comparison of central and peripheral GnRH receptors.

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Receptor expression on cell surface	Dynamic, highest level before LH surge	Dynamic in ovary, depends on degree of follicular development and stage of estrous cycle

- Central GnRH receptor number varies → directly correlated with gonadotropin secretory capacity of pituitary gonadotrophs
- GnRH receptor message levels are regulated by …
  - Suppress: E2, P, gonadotropins
  - Stimulate: E2, calcium, protein kinase C
- Lactation or undernutrition → # of GnRH receptor ↓ dramatically
- exposure to pulsatile GnRH → up-regulation
- exposure to continuous GnRH → down-regulation

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- GnRH receptor expression was greatest in granulosa cells from graafian and atretic follicles with lowest in preantral, small antral follicles and corpus luteum.
- Ex: in atretic follicle → mRNA 3X ↑ on the day of proestrus
- GnRH receptor expression in rat ovary is correlated with expression of pituitary GnRH receptors.
- GnRH and GnRH receptors
  - Not immunostained in primordial to early antral follicle
  - Predominantly in granulosa cells, not theca cells
  - Localized in > 85% ovarian cancer

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- The expression of GnRH and GnRH receptor protein in the human ovary is temporally and spatially specific.

→ supports the physiologic role of an **autocrine regulatory system** involving GnRH and GnRH receptor in follicular development and corpus luteal function.

Physiological functions and mechanism of actions

- Regulation of hCG secretion
- Regulation of implantation
- decreasing cell proliferation and mediating apoptosis in tumor cells

Regulation of hCG secretion

- ***Hypothesis***: placental GnRH may be involved in the auto/paracrine regulation of synthesis of hCG
  - Highest GnRH level in the placenta are present during 1st trimester of pregnancy (= hCG distribution)
  - secretion of hCG from placental cells found to be suppressed by GnRH antagonist
  - fully demonstrated both in vitro and in vivo

- placental cells from anencephalic fetuses compared with normal trophoblastic cell: ↓ binding capacity for GnRH and its agonists and capacity to produce hCG
  - → GnRH secreted from pituitary up-regulates **peripheral GnRH receptors** and stimulates hCG production in the placenta
- GnRH antagonists block both GnRH- and GnRHa induced effect.

- Immunohistochemistry
  - Most intense staining for GnRH → GA 8 wks
  - GnRH mRNA remain **constant** throughout gestation
  - **GnRH receptor** mRNA expressed in both cytotrophoblasts and syncytiotrophoblasts, *changing paralleling the time course of hCG secretion during pregnancy*
- Paracrine / autocrine regulation of hCG secretion by placental GnRH is mediated through an increase followed by a decline in **GnRH receptor gene expression** from 1st trimester to term placenta

Regulation of implantation

- Very Complex !!!
- embryonic-maternal dialogue in ''window of implantation''
- Growth factors and cytokines secreted by developing blastocyst to enhance uterine receptivity
  - direct: down-regulation of cell polarity
  - indirect: stimulate ovarian steroidogenesis through hCG, until placental P production sufficient to maintain pregnancy

- GnRH and hCG were produced in vitro by periimplantation embryos of the rhesus monkey.
  - GnRH secretion before hCG
  - Embryos that failed to hatch and attach secreted **lower** GnRH and hCG.

Seshagiri PB, Hum Reprod 1994;9:1300–7

- GnRH and GnRH receptors present in preimplantation human embryos and fallopian tubes in the luteal phase at both mRNA and protein levels.
- dynamic expression of GnRH and GnRH receptor in both epithelium and stroma of human endometrium
  - highest in luteal phase

→ GnRH as molecular autocrine / paracrine regulator in embrynic – endometrial interactions during early implantation

- During reproductive life, endometrial GnRH may play a paracrine/autocrine role in the early stages of implantation by modulating embryonic trophoblastic secretion of hCG.

Raga F, Biol Reprod 1998;59:661–9

- **GnRH receptor** found to maintain **a constant level** during all developmental embryonic stages → embryo communicates with the maternal tubal epithelium and endometrium through the GnRH system to promote embryonic development and endometrial receptivity

- A Cochrane review of 27 RCTs comparing the GnRH antagonist to the long protocol of GnRHa in ART cycles revealed significantly lower clinical pregnancy, ongoing pregnancy, and live birth rates.
  - impact of GnRH antagonist on endometrium and implantation ?
  - GnRH agonist or antagonist had **no** detrimental effect on trophoblast invasion and stromal cell decidualization in vitro
  - **GnRH receptors** ↑ in decidualized stromal cells

- GnRHa for additional luteal support → potential local effect from endometrial GnRH receptors
- A RCT: 164 pts in GnRH antagonist protocol → ICSI → luteal phase support (P alone or GnRHa + P)
  - GnRHa + P group : ↑ implantation, clinical pregnancy and live birth rates

Isik AZ, Reprod Biomed Online 2009;19:472–7

- GnRH antibody has been described in the maternal circulation of pregnant women with previous misscarriage and low levels of hCG

Cell proliferation and apoptosis

- GnRHa in vitro suppresses the growth of …
  - endometrial cancer, ovarian cancer, and estrogen-dependent and estrogen-independent breast cancer
  - benign tumor leimyoma
- GnRH analogues reduced the # of GnRH-responsive carcinoma cells and their cell lines to 50% - 80%
- Antiserum to GnRH greatly increases the growth of ovarian cancer cells.

Antitumor effect of GnRHa is specific and directed in part through GnRH receptors on membranes of tumor cells.

GnRHa inhibits tumor cell proliferation

- Mechanism: not known
- Fas-Fas ligand → programmed cell death and apoptosis
  - transmembrane proteins of tumor necrosis factor family
  - **Fas**: detected in variety of normal and neoplastic cells
  - **Fas ligand**: activated T cell, tumors (human colorectal cancers, melanomas, HCC, astrocytomas, lung cancers), GnRHa induces Fas ligand expression in GnRH receptor-bearing tumors
  - GnRHa induced apoptosis in leiomyoma cells associated with ↑ expression of Fas and induction of Fas ligand

GnRHa inhibits tumor cell proliferation

- P53 : *transport Fas from the Golgi complex to membrane surface* and then play a role in the induction of apoptosis by combining Fas with Fas ligand
- GnRHa (triptorelin, leuprolide) induce a reversible reduction of cell proliferation → ↑ resting phase G0/G1
- Jun D (transcription factor): negative regulator of cell proliferation → slowed cell growth, ↑ resting phase G0/G1

→ JunD activation by GnRH plays an important roles as a modulator of cell proliferation and cooperates with GnRH

- GnRH may be involved in the process of luteinization and luteolysis.
  - GnRH induces remodeling of the extracellular matrix by **stimulating MMPs** which degrades collagens
  - GnRH-induced apoptosis also contributes to
    - endometriolytic process
    - follicular atresia (↑ GnRH receptor expression)
    - follicular rupture
    - oocyte maturation

Clinical application
cancer therapy

- GnRHa used in treatment of breast cancer, endometrial cancer, ovarian stromal tumor and ovarian epithelial cancer
  - Desensitization or decrease in GnRH receptor in pituitary → ↓ gonadotropin secretion and hormone production
  - directly suppress the growth of endometrial, ovarian, breast cancer and leiomyoma cells in vitro

- GnRHa also inhibit non-reproductive tract tumors, such as melanoma, supports the following speculations:
  - GnRHa might exert a direct anti-proliferative action at the level of tumor
  - Clinical use extended to nonreproductive tissue tumors that express GnRH receptors

- Conjugated compounds
  - GnRHa + cytotoxic radicals: AN-152 (doxorubicin + GnRHa) → selectively destroy cancer cell
  - GnRHa or antagonist + photosensitizer agent
  - Clinical trials needed to verify the effectiveness of GnRH analogue-targeted anticancer treatment
  - potential limitations:
    - Low # of GnRH receptors on tumor cells
    - Poor internalization of GnRH receptos

Prevention of chemotherapy-induced ovarian damage

- Owing to improvements in cancer therapy → cure rates ↑
- Long-term consequences of cancer therapy and impact of life quality are concerned.
- Gonadal failure: major sequence of cytotoxic C/T
- Candidates

Childhood cancers	Breast cancer	Cervical cancer
Benign ovarian disease	SLE	Autoimmune disease
Receiving pelvic radiation	Prophylactic oophorectomy	Hematopoietic stem cell transplantation

- Currently only unequivocal and clinical available option is cryopreservation of fertilized ova or embryos after IVF and before C/T
- Mature and immature oocyte cryopreservation, ovarian tissue cryopreservation, in vitro oocyte maturation, and human ovarian transplantation → still experimental
- ***Hypothesis***: ovarian suppression can be protective!!
  - Protective role of GnRH analogue treatment against C/T-induced gonadal damage
  - Ataya et al.: GnRH analogue protected ovary against cyclophosphamide in Rhesus monkeys by significantly ↓ the total amount of follicle loss during C/T and daily rate of follicular decline

- 111 pts with Hodgkin lymphomas treated with C/T followed for 2–15 years
  - Received a **monthly injection of GnRHa**, administered before starting C/T until its conclusion, up to a maximum of 6 months → 63/65 (96.9%) pts resumed ovulation and regular menses compared with 63% of 46 control subjects

Blumenfeld Z, Fertil Steril 2008;89:166–73

- 4 phase II studies in premenopausal breast cancer pts → GnRHa cotreatment enables resumption of ovarian function in high percentage of treated pts (83-96%)
  - All 13 pts (26-39 y/o) resumed normal ovarian function after a mean of 4.9 months after C/T

Fox KR, Proc Am Soc Clin Oncol 2003;22:13

  - 86% of 64 pts (27-50 y/o) resumed regular MC despite a relatively advanced median age of 42 years

Recchia F, Anticancer Drugs 2002;13:417–24

non-cancer disease

- 40 young women with **severe SLE** treated with cyclophosphamide
  - Premature ovarian failure (POF) develop in **1/20 (5%) treated with GnRHa** compared with 6/20 (30%) in control group after a minimum of 3 years' follow-up
  - 1st study to analyze a large group of lupus pts with control subjects individually matched for age and cumulative dose of C/T
  - Add-back E2 therapy → protective effect not merely the result of hypoestrogenic environment

Somers EC, Arthritis Rheum 2005;52:2761–7

- Conclusion from a meta-analysis with 9 studies (366 women)
  - GnRHa improve ovarian function and the ability to achieve pregnancy after C/T
  - in pts with GnRHa treated during C/T:
    - associated with a 68% increase in rate of preserved ovarian function (RR= 1.68【95% CI 1.34-2.1】)
    - 22% achieved pregnancy (14% in control group, RR= 1.65【95% CI 1.03-2.6】)

Clowse ME, J Womens Health (Larchmt) 2009;18:311–9

RCT #1

- 80 pts (18-40 y/o) with early-stage breast cancer after OP, after 8 months follow-up

	Resume mense	POF (not defined)
C/T alone	33.3%	66.6%
C/T + goserelin	89.6%	11.4%

- The incidence of amenorrhea and POF in control group were significantly higher than previous data.
- Baseline E2 and FSH levels statistically different in 2 groups
- Estrogen receptor status and tamoxifen usage not controlled
- Length of follow-up from initiation or completion of C/T ?

RCT #2

- Small, prospective
- Only RCT demonstrated that GnRHa was **NOT** effective in preserving fertility in pts receiving C/T for Hodgkin disease

3 year f/u	♀ amenorrhea	♂ oligo/azoospermia
buserelin before and during C/T	4/8	30/30
control	6/9	

- phase III RCTs
- Ovarian protection → preserve future fertility, prevent osteoporosis and ovarian failure

Mechanism of protective effect of GnRHa

- Unclear
- Murine ovaries contain GnRH receptor → protective effect mediated by direct effect of GnRHa on ovaries
- Knock-down of GnRH receptor expression, doxorubicin-induced apoptosis in human endometrial and ovarian cancers and in human breast cancer cell line (MCF-7) ↑

→ Peripheral GnRH receptor activation suppressed chemotherapeutic drug-induced apoptosis in these cancers

- E2 productivity of GnRH receptor(+) granulosa cells during exposure to doxorubicin + GnRHa in culture
  - GnRHa may retard doxorubicin-induced granulosa cell damage
  - → GnRHa protects the gonads during chemotherapy may be through local GnRH receptor–mediated mechanism
- GnRHa may preferentially steer cells into cell cycle arrest (G0/G1 phase)
- Hypogonadotropic state → ↓ ovarian blood flow → ↓ in the exposure of ovaries to C/T
- GnRHa may up-regulate an intragonadal anti-apoptotic molecule

GnRH antagonist

- Little clinical data on GnRH antagonist for prevention of C/T associated gonadotoxicity
  - conflicting data in animal studies → due to differences in species and/or dosing regimens

Summary (I)

- GnRH receptors were found in a variety of normal and tumoral human (non-)reproductive tissues.
- Peripheral GnRH/GnRH receptor system:
  - autocrine-paracrine regulator
  - have important physiologic functions
  - regulators of hCG synthesis and implantation
  - play crucial roles in antiproliferation and apoptosis

Summary (II)

- GnRHa have been used in cancer treatment and ovary protection during chemotherapy
  - taking advantage of the local direct effect mediated by peripheral GnRH receptors
- Further research to clarify functions of peripheral GnRH receptors may lead to discovery of new therapeutic options.

Thank you !