Steroidogenesis-Inducing Protein: An enigmatic protein with multiple biological functions

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Clark Atlanta University, Atlanta.
Research Projects

• TGFβ in prostate cancer: intracellular signaling mechanisms involved in TGFβ effects on proliferation, invasion and metastasis of prostate cancer cells.

• Mechanisms involved in development of androgen independence in advanced stages of prostate cancer.

• Role of SIP in prostate cancer.
Integration of Biological Functions by Chemical Messengers

**ENDOCRINE**

Gland

Target cell

**PARACRINE**

Cell A

Cell B

**AUTOCRINE**

Cell
Regulation of Reproductive Development and Function

- Hypothalamus
  - GnRH

- Pituitary
  - FSH
  - LH

- Testis
  - T
  - Growth Factors
  - Cytokines
  - Gonadal peptides
  - Sperm

- Ovary
  - E2, P
  - Oocyte

- Oocyte
Paracrine and Autocrine Regulation of Testis

**Experimental Approaches**

1. Effects of known growth factors and cytokines on testicular cells.
2. Identification of novel bioactive peptides and proteins in testicular secretions.
Follicular Development in the Human Ovary

Follicular Fluid Proteins

1. Steroidogenesis
2. Proliferation
3. Luteinization and Luteal Function
Effects of hFF on Steroidogenesis in Leydig Cells

Leydig Cells (60-day old rats)

- Incubated in the presence of hCG, hFF and serum proteins for 3 hours.

- Determination of testosterone by RIA.

Graph showing the effects of hCG and serum on testosterone production.
LH stimulated testosterone production in Leydig cells

LH

StAR

Mitochondrion

P450scc

Free Cholesterol

ATP

cAMP

Transport

Pregnenolone

3βHSD

Progesterone

17α-Hydroxylase

17α-Hydroxyprogesterone

C17-20Lyase

Androstenedione

17KSR

Testosterone

P450 arom

Estradiol

Cell membrane

Cell membrane
Effects of cycloheximide and aminoglutethemide on SIP stimulated steroid production

- AMG cont.
- SIP cont.
- LH + AMG
- LH + CH
- LH
- SIP + AMG
- SIP + CH
- SIP
- Control

Pregnenolone (pmol/ 500,000 cells)
Effects of hFF proteins on Steroidogenesis in Rat Adrenal cells
Physico-chemical Properties of Steroidogenic Factors in hFF

Gel Chromatography

Isoelectrofocusing
• Steroidogenesis - Inducing Protein (SIP)
Life History of Leydig Cells

Leydig Cell Number

Fetal
Adult
Stages of Leydig Cell Development

1. Immature Leydig cell
2. Adult Leydig cell

Birth, Day 7, Day 14, Day 21, Day 28, Day 56
SIP Stimulates DNA Synthesis in Rat Leydig Cells

Immature rat Leydig Cells
Cultured with different doses of SIP for 18 h
Washed and incubated with $^3$H-thymidine for 4 h
Determined incorporation of $^3$H thymidine into DNA

![Graph showing the effect of SIP on DNA synthesis]

- **SIP (μg)**: 0, 2, 5, 10, 25, 50
- **$^3$H-Thymidine (dpm x 10^3 /μg protein)**: 0, 4, 8, 12
Autoradiography of labeled Leydig Cells

(a) C
(b) IL-1
(c) SIP
(d) IL-1 + SIP
1. Effects of SIP on proliferation of rat granulosa cells.

2. Effects of SIP on proliferation of human ovarian epithelial cancer cells.
SIP Interacts with TGFβ to Stimulate DNA Synthesis in Rat Granulosa Cells
Ovarian Epithelial Cancer

- **INCREASED RISK ASSOCIATED WITH**
  1. NUMBER OF OVULATIONS
  2. USE OF FERTILITY DRUGS

- **REDUCED RISK ASSOCIATED WITH**
  1. MULTIPLE PREGNANCIES AND LACTATION
  2. ORAL CONTRACEPTIVES
Ovarian Epithelial Cancer Cell Lines

1. HEY CELLS
2. OVCAR- 3 CELLS
3. CAOV-3 CELLS
4. SKA CELLS
5. MLs
6. OW-1
7. Sau
Effects of SIP on DNA Synthesis in Ovarian Surface Epithelial Cell Lines

- MLS
- SAU
- OW-1
- Ska

- CaOV-3
- OVCAR3
- HEY

3H-thymidine (cpm x 10^{-3}/well)

CONT
SIP

CONT
SIP
Biochemical and Molecular Characterization of SIP
Purification and Amino Acid Sequence of SIP

EVQLVESG Peptide 1
DVNGGGATLPQPLYQTA Peptide 2
FNWYVDGVEVHNAK Peptide 3
Conserved SIP Peptide Sequences Found in Protein Databases

Peptide 1

33.8 %

Peptide 2

11.4 %

Peptide 3

97.4 %
## Homology of SIP with other Human cDNAs

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<tr>
<th>Accession number</th>
<th>Tissue/Cell Source</th>
<th>Function</th>
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<td>BAC05017</td>
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## DING Proteins

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<td><strong>SIP</strong>, human Steroidogenesis-Inducing Protein, 50KD</td>
<td>Human Follicular Fluid; Human Granulosa-Luteal cells; Human Ovarian Epithelial Cancer Cells</td>
<td>DVNGGGATLPQPLYQTA</td>
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<tr>
<td><strong>p205</strong>, human synovial stimulatory protein</td>
<td>Rheumatoid Arthritis Synovial Fluid</td>
<td>DINGGGATLPQPLYQTAAVLTAGFA</td>
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<td><strong>HSFP</strong>, 40KD protein</td>
<td>Human fibroblast cells, cervical carcinoma cells</td>
<td>DINGGGATLPQPLYQTSGVLTAGFAPYTG</td>
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<td><strong>CAI</strong>, Crystal Adhesion Inhibitor, 39KD</td>
<td>Renal epithelial cells</td>
<td>DINGGGATLPQPLYQTSGVLTAGFAPYISVNAK</td>
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<td><strong>p40</strong>, cotinine receptor</td>
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## Comparison of SIP and P205 Sequences

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<td>SIP</td>
<td>EVQLVESG</td>
<td>DVNGGGATLPQPLYQTA</td>
<td>FNWYVDGVEVHNAK</td>
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**p205**

- Peptide 1: XVQLVE
- Peptide 2: DINGGGATLPQPLYQTAGVLTAGFA
- Peptide 3: YVDGVEVHNAK

**SIP**

- Peptide 1: EVQLVESG
- Peptide 2: DVNGGGATLPQPLYQTA
- Peptide 3: FNWYVDGVEVHNAK
Anti-p205 Cross-react With Bioactive SIP

EVQLVESG
SIP production and effects on cancer cells

DU145   LNCaP   PC3    PC3M
β-Actin

PC3M DU145 MCF7 HEY HepG2 S-200

3H-Thymidine Incorporation (CPM/Well)

CONTROL   EGF   SIP
Mechanism of Action of SIP
SIP-induced Tyrosine Phosphorylation in immature rat Leydig cells
Mechanism of SIP Action

SIP

Genistein

Ras/Raf

MEK1/2

Erk1/2

PI3K

PKB/Akt

steroidogenesis

Cell survival, proliferation
Genistein Blocks the Effects of SIP on DNA Synthesis and Steroidogenesis in Leydig Cells

DNA Synthesis

Steroidogenesis

$^{3}$H-thymidine (cpm/well)

Testosterone (ng/10$^6$ cells)
Mechanism of SIP Action

- **SIP**
- **Ras/Raf**
- **MEK1/2**
- **Erk1/2**
- **PI3K**
- **PKB/Akt**

**Compounds:**
- PD98059
- U0126
- Genistein
- LY294002

**Major Processes:**
- Steroidogenesis
- Cell survival, proliferation
Ras-Raf-MAP Kinase signaling pathway
SIP effects on the activation of MAP-Kinase in ILC cells

- Ras-GTP
- p-MEK
- MEK
- p-ERK1
- p-ERK2
- Actin
- Pan-ERK
PI3 Kinase-PKB/Akt Signaling Pathway
SIP effects on the activation of Akt in ILC cells
Mechanism of SIP Action

Ras/Raf → MEK1/2 → Erk1/2 → PKB/Akt → Cell survival, proliferation

SIP

Genistein

PD98059, U0126

LY294002

steroidogenesis
Effects of SIP on DNA Synthesis and Steroidogenesis in Leydig Cells in the Presence of MEK and PI3-Kinase Inhibitors
PKA signaling pathway is involved in SIP effects on steroidogenesis in ILC

Testosterone Production in ILC

Testosterone (pg)

Control  H89,5uM  H89,10uM

C  LH  EGF  SIP
## SIP Effect on Steroidogenic Effectors in ILC

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<tr>
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Mechanism of SIP Action

SIP

Ras/Raf → MEK1/2 → Erk1/2 → PKB/Akt → Cell survival, proliferation

Genistein

PD98059, U0126

LY294002

Steroidogenesis
SIP peptide sequences

Peptide 1: EVQLVESG
Peptide 2: DVNGGGATLPQPLYQTA
Peptide 3: FNWYVGDGVEVHNACK
Mitogenic effects of synthetic SIP peptide on DNA synthesis in ILC

SIP Effects on DNA Synthesis in ILC

3H-Thymidine (cpm/well)

C  LH  EGF  S200  HEP  P
Mitogenic effects of synthetic SIP peptide in HEY cells

SIP Effect on DNA Synthesis in HEY Cells

3H-Thymidine (cpm/well)

C, 5% FBS, S200, HEP, P-5ng/ML, P-10ng/ML, P-20ng/ML, P-50ng/ML
Synthetic SIP peptide activates MAP-kinase and PI3-kinase/Akt pathways in ILC

5 min

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30 min

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SIP peptide effects on steroidogenesis in ILC

SIP peptide effects on Androgen production in ILC

Testosterone (pg/10^6 cells)

C6h, LH6h, SIP6h, P10.6h, C24h, LH24h, SIP24h, P10-24h, C48h, LH48h, SIP48h, P10-48h
### Synthetic SIP peptide Effects on Steroidogenic Effectors in immature rat Leydig cells

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### Cytoskeleton

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Conserved SIP Peptide Sequences Found in Protein Databases

<table>
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<th>Peptide 1</th>
<th>Peptide 2</th>
<th>Peptide 3</th>
</tr>
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<tbody>
<tr>
<td>33.8 %</td>
<td>11.4 %</td>
<td>97.4 %</td>
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Peptide 1

Peptide 2

Peptide 3
Multiple Sequence Alignment

BAC04926
BAC05186
AAH06402
BAC05017
CAA75030
BAC05421
BAC05203
AAH14667
AAO17823
AAH18747
AAH41037
SIP

..EVELVESG...AKGNQPRVDIVAS.......IEN...FNWYVDGVEVHNAK...
..EVQLVESG...ARGYSTAAAIKYN.....WFDS...FNWYVDGVEVHNAK...
..EVQLVESG...AKDIELDIVVVPPAPMRGSLGFDY...FNWYVDGVEVHNAK...
..QVQLVQSG...ARDTTAYFDDAGYRP...YYFDL...FNWYVDGVEVHNAK...
..DVQLVESG...ARGTRFLELTS.......RGQMDQ...FNWYVDGVEVHNAK...
..EVQLLDGS...AKDSSCSTSACYD.......HF...FNWYVDGVEVHNAK...
..EVLLLESG...AHAFDVLRYFHRGFY......FAH...FNWYVDGVEVHNAK...
..EVQLVESG...NTDARSVGSLEWPNNYHG...MNV...FNWYVDGVEVHNAK...
..EVQLLESG...AKDREVTMIVVPLGG......FDY...FNWYVDGVEVHNAK...
..EVQLVESG...ARDGSS.........WY.RDWFD...FNWYVDGVEVHNAK...
..EVQLVESG...AREFESTMVTNADYY.YFYMDV...FNWYVDGVEVHNAK...
..EVQLVESG...Variable region ...FNWYVDGVEVHNAK...

1 3
• We have isolated and characterized a novel gonadal protein, SIP, which stimulates steroidogenesis and proliferation of gonadal cells.

• SIP belongs to a family of recently recognized human proteins. SIP shows significant homology with a protein isolated from synovial fluid which stimulates proliferation of T-lymphocytes.

• SIP is produced by granulosa cells during normal ovarian cycle and may play a significant physiological role during follicular development, ovulation, luteinization and/or maintenance of luteal function.
• Presence of SIP in ascites fluids from ovarian cancer patients, its secretion by ovarian cancer cells, and its stimulatory effects on proliferation of ovarian epithelial cancer cells suggest that SIP may play a role in the etiology of ovarian cancer.

• SIP is produced by prostate cancer cells and it stimulates proliferation of these cells indicating that SIP is an autocrine growth factor for these cells.

• SIP exerts its effects on target cells via a tyrosine-kinase signaling pathway. SIP effects on cell proliferation are mediated by MAP-kinase and PI3-kinase/Akt pathways.
Acknowledgments

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Jay Reddy
Ozlen Konu
Zhiqiag Cheng
Ana Cecilia Millena
Miao Zhang

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Brandt Schneider