Functional Analysis of ER Transcriptional Inhibition by CtIP Co-repressor Complex

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Estrogen Receptor (ER)

• *ER belongs to family of proteins called nuclear receptors*
  – *Ligand initiated transcription factor*

• There are numerous nuclear receptor motifs that interact with ER, such as AP-1 and SP-1
Estrogen and Breast Cancer

• Breast cancer was first recognized to be an E2-dependent disease in 1896.

• Prolonged exposure to E2 results in increased risk for breast cancer.

• E2 promotes breast cancer progression by stimulating malignant cell proliferation
Generalized ER Regulated Transcription

Conformation Change → Estrogen induced transcription

Conformation Change → Antagonistic inhibition of transcription
Therapy for Breast Cancer

• Blocking the action of E2 on its receptor

• Reducing circulating levels of E2
Tamoxifene

• Tamoxifene blocks the effects of E2 by binding to ER

• Conformation change = change in co-regulators
Suspected Mechanisms of Resistance

- Loss of expression or mutation of ER
- Altered expression of co-regulator proteins.
Tamoxifen Resistance

- Many tumors that respond to TAM eventually develop resistance.
- Mechanisms of resistance are still poorly understood.
Development of Tamoxifen Resistant MCF-7 Breast Cancer Cell Line Variants

MCF-7
IMEM (w/o Phenol Red)
5% FBS
+ 4-OH-TAM
>2 years

TAMR1
IMEM (w/o Phenol Red)
5% FBS
4-OH-TAM

TAMR2
IMEM (w/o Phenol Red)
5% CSS
4-OH-TAM

A

B
Serial Analysis of Gene Expression (SAGE)

- SAGE in TAMR1 and TAMR2 identified many dysregulated genes
- CtIP was most significantly down regulated
Loss of CtIP in MCF-7 Tamoxifen Resistant Cell Lines
Tamoxifen Resistant MCF-7 Cell Lines
Observation

• Developed Tamoxifen resistance by breast cancer cells

• CtIP most significantly down regulated protein
Hypothesis

• BRCA1 and CtIP are components of a multiprotein complex that functions as a corepressor of ER transcriptional activity.

• Disruption of this complex will result in increased transcription of ER-dependent genes in the absence of E2 stimulation.
Does tamoxifen act as an agonist in the absence of corepressors?
Investigation

• Experiments and Techniques
  – Growth Curve Determination (MTT Assay)
    • Metabolism of reagent by growing cells results in measurable change in absorbance
    • Change in absorbance is proportional to cell number
  – B-gal/Luciferase Assay
    • Utilizes ER-regulated firefly luciferase and beetle luciferine to generate luminescent signal
    • Measured luminescence indicates level of transcription
  – RT-PCR Gene Expression Assay
    • RT-PCR provides a quantitative approach to identifying relative levels of mRNA product
    • Allows us to measure differential gene expression within cells
MTT Growth

- Insert graph showing tamr1 vs mcf-7 in css/tam, tamr1 css/tam vs. tamr1 css/etoh, this shows that tam is not acing as agonist in absence of CtIP it just lost its antagonistic properties.

- Show tamr1 in E2/tam/css vs. tam to show tam successfully blocks effects of E2
Luciferase Reporter System

- Luciferase Assay - used to quantify ER-regulated gene expression

Transfection of Plasmids

B-gal
ERERE-Luc
ERa

*Mutant CtIP

TAMR1 Cell
Expression of Transient CtIP

CtIP Variant

ΔPLDLS  S327A  FL3-CtIP  Empty Vector

Actin
Treatments

- 1uM TAM
- 10nM E2
- 1uM TAM/10nM E2
- EtOH
Results
RT-PCR Gene Expression Assay

• Methods and Conditions
• Results(?)
Interpretation of Data

• Growth Curve Determination
  – Interpretation of Data
Interpretation of Data

• B-gal Luciferase Assay
  – Interpretation of Data
Interpretation of Data

• RT-PCR Gene Analysis
  – Interpretation of Data
Conclusion
CtIP Repression of Estrogen Dependent Transcription

+CtIP
-CtIP

Fold Stimulation

Treatment

TAM
E2
TAM/E2
Fold Activation Over Vehicle

Transfections

Estrogen Induced Transcription

CtIP

No CtIP

PLDLS

S327A
Estrogen to Vehicle Comparison

Transcription

Transfections

CtIP +E2
CtIP -E2
No CtIP +E2
No CtIP -E2
PLDLS +E2
PlDLS -E2
S327A +E2
S327A -E2
Growth Curve Assay

Absorbance vs. Number of Assays for different cell lines and conditions:
- TAMR1 TAM/FBS
- TAMR1 TAM/CSS
- TAMR1 ETOH/FBS
- TAMR1 ETOH/CSS
- TAMR1 E2/CSS
- TAMR1 E2/TAM/CSS
- MCF7 TAM/FBS
- MCF7 TAM/CSS
- MCF7 ETOH/FBS
- MCF7 ETOH/CSS
- MCF7 E2/CSS
- MCF7 E2/TAM/CSS