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Measurement of epigenetic alterations from patient's tissues in myoma, adenomyoma and endometriosis

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Measurement of epigenetic alterations from patient's tissues in myoma, adenomyoma and endometriosis

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Abstract

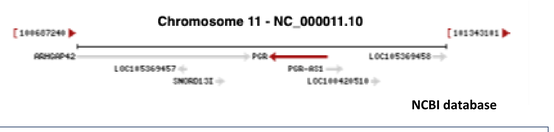
Background: Myoma, adenomyoma, and endometriosis are estrogen-dependent gynecologic diseases and result in reproductive dysfunction and pelvic pain in women. However, these gynecologic diseases have a complex and poorly understood etiology, involving both genetic and environmental factors. Epigenetic alterations, heritable changes that can modify gene expression without affecting genetic sequence, are associated with the development and progression of numerous pathological states and diseases. Therefore, there is great potential for the use of epigenetics as biomarkers to better understand the early-stage biological responses and molecular mechanisms of gynecologic diseases. We aimed to examine levels of global DNA and gene-specific methylation, which are epigenetic alterations that could be associated with development of gynecologic diseases, including myoma, adenomyoma, and endometriosis.

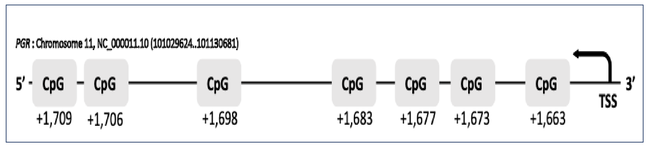
Methods: We measured global DNA methylation (LINE-1) as well as disease relevant gene-specific methylation (i.e. ER, PR, and aromatase) using pyrosequencing assay. For this measurement, gene-specific primers for the selected genes were designed using the Pyro-Mark assay design software. Genomic DNAs from each tissue were extracted, and underwent bisulfite modification to convert unmethylated cytosine residues to uracil. A Pyromark Q96 MD was used for all subsequent pyrosequencing. Samples were processed in duplicates on plates with water controls. Percent methylation of a sample was calculated by averaging all of the interrogated CpG sites.

Results: Different methylation levels of selected genes were measured from myoma, adenomyoma, and endometriosis tissues. Our obtained results suggest that epigenetic changes are involved in development of different types of gynecologic diseases.

Primer design & validation & PCR

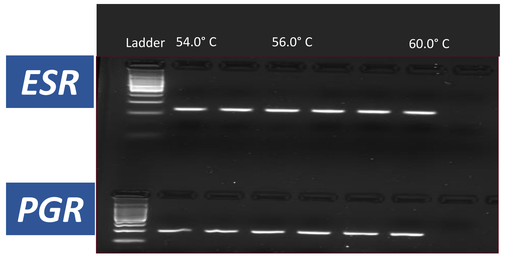
PGR



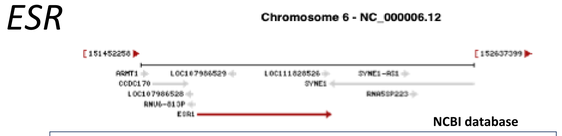


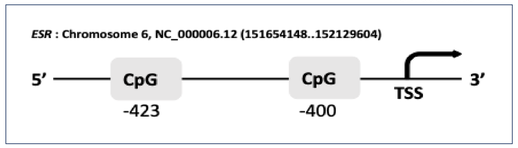
Progesterone Receptor gene (*PGR)* is located on human chromosome 11. Using NCBI and Ensembl databases, seven CpG sites were selected for primer design sequencing

A gradient PCR was used to determine ideal PCR thermocycler conditions for each primer set prior to conducting pyrosequencing experiments



ESR





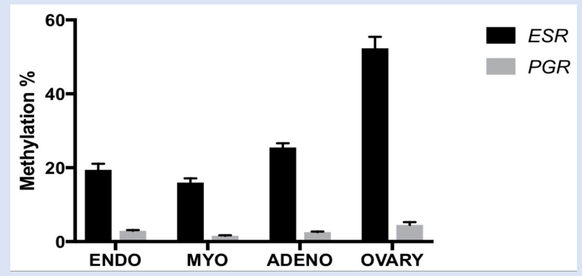
Estrogen receptor gene (*ESR)* is located on human chromosome 11. Using NCBI and Ensembl databases, two CpG sites were selected for primer design sequencing.

Target (Gene ID)	Primer	Sequence (5'-3')
PGR	Forward: Reverse: Sequencing:	GGTTTTGTTAGGGATAGGATTT CTACCTCCAACACCCCTTATA[a] TGTTAGGGATAGGATTTTT
ESR	Forward: Reverse: Sequencing:	AAGGGTTGAAGAGTGTGAGAA ACTCAAAAACCCCCTCTACACTTCTT[a] AGTGTGAGAAGTTAGAT

[a]Biotin labeled primer

Primer sequences were determined using PyroMark Assay Design software.

Preliminary Epigenetic Results



Promoter methylation status of DNA from different gynecologic tissue. n=9-11 per tissue-type. Data expressed as mean ± SEM

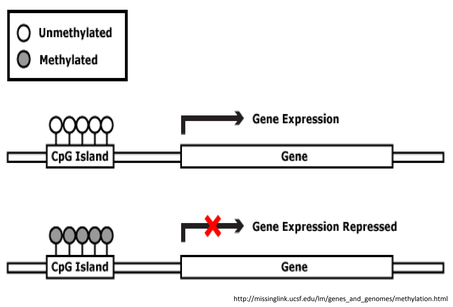
Conclusions

- **This study, using DNA methylation methods including Bisulfite conversion, PCR and pyrosequencing on a Pyromark-Q96, supports that epigenetic changes could be involved in development of different types of gynecologic diseases.**
- **Promoter methylation of *PGR* and *ESR* appears to be tissue-specific in different gynecologic tissue DNA.**
- **These epigenetic differences could be useful in biomarker development.**
- **These epigenetic changes could have a mechanistic influence on corresponding gene expression**
- **Further investigation of these methylation patterns is needed.**

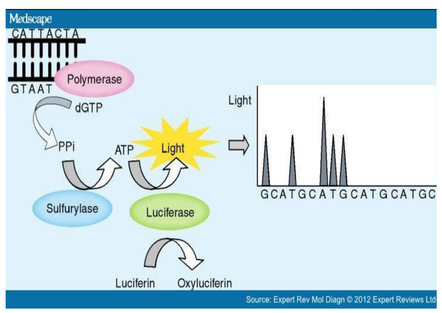
INTRODUCTION

HYPOTHESIS

Tissue-specific promoter methylation patterns will be observed in DNA of gynecologic tissue of endometriosis patients.



Methylation within gene promoter regions can influence levels of gene expression.

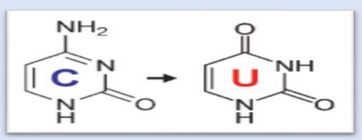


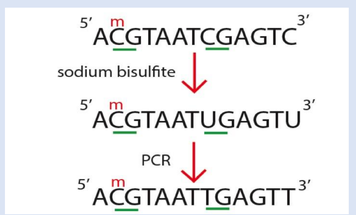
Pyrosequencing using an ATP-driven light reaction to generate a pyrogram used to measure % methylation.

METHODS

DNA Isolation → Bisulfite Conversion → Primer Design & Validation + PCR → Pyrosequencing → Data analysis & Interpretation

Bisulfite conversion

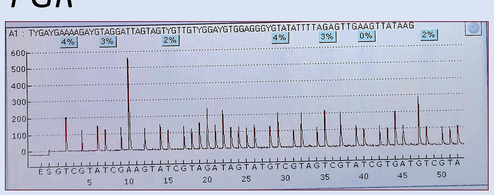




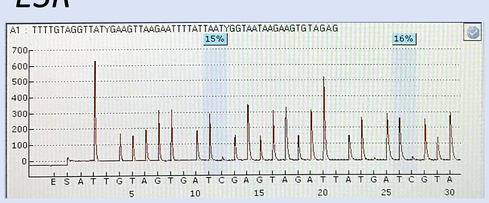
Bisulfite conversion is an experimental method to distinguish methylated from non-methylated cytosines through conversion of un-methylated cytosines to uracil.

Pyrosequencing Pyrograms

PGR



ESR



Representative pyrograms for *PGR* and *ESR*. CpG % methylation is averaged across all CpG sites per assay.

Future Directions

- There is a potential clinical application of these findings as biomarkers of gynecologic disease.
- Global methylation measurement as an overall biomarker determination
- Measurement of promoter methylation in other pro-inflammatory and gynecologic related genes.
- Measurement of global and gene-specific methylation in blood.
- Isolation of RNA from tissues to conduct gene expression experiments.