ANALYSIS OF THE DIFFERENTIAL EXPRESSION OF MICRORNA LET-7A AND ITS RELATIONSHIP TO THE MOLECULAR SUBCLASSIFICATION OF BREAST CANCER IN A COHORT

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Breast Cancer

Heterogeneous group of disease which is govern by followings hallmarks
Incidence and mortality of Breast cancer

According to Punjab Cancer Registry report of 2014, 1393 (44.3%) women were diagnosed with breast cancer in Punjab area.
Histological Diagnosis

Cancer affecting the inner lining of milk ducts

Ductal Carcinoma

Cancer affecting the part of the breast that produce milk i.e. lobules

Lobular Carcinoma

Based on tissues

Types of Breast Cancer

Based on invasiveness

Noninvasive Carcinoma

Also known as “In-situ”

Invasive Carcinoma

Cancer spreading to other parts of breast from ducts or lobules

Lobular Carcinoma

In-Situ (LCIS)

Cancer not spreading to other parts of breast

Ductal Carcinoma

In-Situ (DCIS)

Based on hormones and genes

ER-Positive Breast Cancer

Estrogen receptor sensitive breast cancer

HER2-Positive Breast Cancer

Cancer as a result of HER-2 gene, which is responsible for growth, division and repair of cells
Molecular Diagnosis

- luminal A, (ER + & low grade)
- luminal B, (ER + but high grade)
- tumor enriched with human epidermal growth factor receptor 2 (Her2),
- basal-like (ER-, PR-, HER2/ Nue -)
[Research Query]

- Breast Cancer had been studied extensively at the molecular level but most of the molecular mechanisms underlying its progression and metastasis remain poorly understood.

- This has led to a significant interest in the quest for novel predictive markers for breast cancer.

- Intensifying research in miRNA studies has resulted in the identification and confirmation of aberrant miRNA expression in a number of human diseases including Breast Cancer.
Micro RNA

- miRNAs are endogenous, noncoding small RNAs with 20–25 nucleotides in length.

- They play an important regulatory role through complimentary binding of the 3' untranslated regions (UTRs) of target genes thus resulting in the degradation of the target mRNA and inhibition of translation.

- The **oncogenic miRNAs (oncomiRs)** display anti-apoptotic activity and are over expressed in cancer cells.

- In contrast, miRNAs with anti-proliferative and proapoptotic activity function as **tumor-suppressors** and are under expressed in cancer cells.

- Different types of cancers at different developmental stages display unique expression profiles of different microRNAs.
Biogenesis of microRNA

1. **Transcription**
   - microRNA gene or intron
   - RNA Pol II / III

2. **Cleavage**
   - pri-microRNA
   - Drosha, DGCR8

3. **Nuclear export**
   - Exportin-5, Ran

4. **Cleavage**
   - Dicer, TRBP
   - microRNA duplex

5. **RISC formation**
   - Ago2
   - Mature microRNA

6. **mRNA target cleavage**, **Translational repression**, **mRNA deadenylation**

7. **Degradation**
miRNA in Breast Cancer

Tumor suppressor
- Mainly targets the oncogenes involved in proliferation, invasion, apoptosis, and metastasis
  - e.g., let-7 family, miR-34b, miR-145, miR-205, miR-200 family

Oncogene
- Target genes involved in proliferation, invasion, angiogenesis, apoptosis
  - e.g., miR-10b, miR-21, miR-17-92 cluster

Chemoresistance/chemosensitivity
- Targets genes involved in multidrug efflux pump, e.g., MDR1
  - e.g., miR-19, miR-21, miR-34a, miR-203, miR-221/222, miR-326

Diagnostic marker
- Serum miRNAs may be used as early marker for breast cancer
  - e.g., miR-21, miR-29a, miR-210 family

Ref: Yin-Yuan et al, Jour of Cancer Biology & Therapy, 2013.14:3, 201-212
Let-7 family of miRNA

Let-7a in Breast Cancer??

Angiogenesis, Cell Adhesion & Migration
- Autotaxin
- Angiogenin
- Integrin

STOP
- RAS
- SKP2
- MYC
- Mitotic Signaling

STOP
- CDKs
- CDC25
- Cyclins
- HMGA2
- Cell Cycle

a, l, c, d, e, f, g, i
OBJECTIVE OF THE STUDY

- To evaluate the miRNA let 7a expression in breast cancer and fibroadenoma

- To evaluate the expression of Let-7a in Relationship To The Molecular Sub Classification Of Breast Cancer
Material & Method
**Inclusion Criteria:**

- Histologically confirmed diagnosed cases of breast cancer
- Females
- Ages Eligible for Study: 30 Years to 65 Years

**Exclusion Criteria:**

- Males
- Patients <30 or >65 of age
- History or presence of other cancers
Extraction of Total nucleic Acid
Ambion (ThermoFisher Scientific) (According to manufacturer instructions)

RNA purity was assessed using a Nanodrop 2000

Preparation of cDNA
ThermoFisher Scientific

qRT-PCR
Taq man Advanced miRNA kit
Assay I.D 478575

Analysis of product

FFPE Blocks

20 μm Section

Preparation of Slides

IHC Staining ER-Alpha, Beta AR, HER2/Nue

Analysis of slides by pathologist
Results
Histological appearance of Fibroadenoma

- Nodular and encapsulated
- Epithelial proliferation appears in a single terminal ductal unit
Histological appearance of IDC

- Desmoplastic reaction in stroma
  (abundant fibrosis and collagen deposition)
- Round to oval nuclei with few mitoses
- Tumor necrosis
Molecular Sub Classification of Breast Cancer

Molecular sub classification Percentage of patient

- LuminalA: 23%
- Luminal B: 33%
- Her 2-Nue: 38%
- TNB: 5%
Immunohistochemistry (IHC) on tissue samples
Luminal A

Estrogen Receptor $\alpha$ 20x

 HER2Neu 20x

ER-$\alpha$ +

HER2/Nue -
Luminal B

Estrogen Receptor $\alpha$  
A

HER2Neu  
B

ER-$\alpha$ +  

HER2/Nue +
Her 2 enriched

Total Cases
No of cases
Axis Title

Triple Negative

Total Cases
No of cases
Axis Title

Total Cases
TNB Cases
<table>
<thead>
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<th>Estrogen Receptor $\beta$</th>
<th>20x</th>
<th>Androgen Receptors</th>
<th>20x</th>
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<td><img src="image2.png" alt="Image B" /></td>
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Estrogen Receptor-$\beta$ +  
Androgen Receptor +
ER-β and AR Expression

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<th>ER-β</th>
<th>Percentage</th>
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<tr>
<td>Positive</td>
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<table>
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<tr>
<th>AR</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Positive</td>
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Expression of Let-7a
Let7a is decreased in invasive ductal carcinoma (IDC) but over expressed in Fibroadenoma.
Let 7a down regulation in high grade & stage II and III IDC
IDC and FA showed inverse expression of let-7a with AR and ER-beta receptor staining

### Let-7a Expression in IDC

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<th>Let-7a Expression in IDC</th>
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<td>No of cases</td>
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<tr>
<td>AR Positive</td>
<td>34</td>
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<tr>
<td>AR Negative</td>
<td>34</td>
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<tr>
<td>ER Positive</td>
<td>34</td>
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<tr>
<td>ER Negative</td>
<td>34</td>
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### Let-7a Expression in Fibroadenoma

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<th>Let-7a Expression in Fibroadenoma</th>
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<tr>
<td>ER Positive</td>
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<tr>
<td>ER Negative</td>
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Conclusion

- Let 7a down regulation seen in cases of invasive ductal carcinoma

- We identified that let-7a down regulation directly correlates with increase grade of invasive ductal carcinoma

- In Benin breast cancer disorder like fibroadenoma shows increase microRNA let-7a expression.
  - let 7a expression is a predictive of molecular subtyping in breast cancer

- Inverse correlation of miRNA let-7a exist in breast cancer related to ER-β and AR

- Altered let-7 family expression is likely to contribute to cancer formation and also progression
Future Prospects of the study

- Use of these unique microRNA expression patterns as tumor diagnostic and prognostic tools, but also for future microRNA gene therapy
- We will try to increase no of cases and controls
- We will diversifies group by including's of others types of breast cancer
- We will include other types of let-7a family member to device molecular signature
THANK YOU