Multiplatform molecular profiling identifies potentially targetable biomarkers in malignant phyllodes tumors of the breast

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Abstract (updated)

Background: Malignant phyllodes tumors are a rare breast malignancy with sarcomatous overgrowth and with limited effective treatment options for recurrent and metastatic cases. Recent clinical trials indicated a potential for anti-angiogenic, anti-EGFR and immunotherapeutic approaches for patients with sarcomas, which led us to investigate these and other treatable pathways in malignant phyllodes tumors of the breast.

Methods: Thirty-six malignant phyllodes tumors (including 8 metastatic tumors with two cases having matched primary and metastatic tumors) were profiled using gene sequencing, gene copy number analysis, whole genome expression, and protein expression.

Results: Whole genome expression analysis demonstrated consistent over-expression of genes involved in angiogenesis including VEGFA, Angiopoietin-2, VCAM1, PDGFRα, and PTGIG. EGFR protein overexpression was observed in 26/27 (96%) of cases with amplification of the EGFR gene in 8/24 (33%) cases. Two EGFR mutations were identified including EGFRVIII and a presumed pathogenic V774M mutation, respectively. The most common mutations were identified including EGFRvIII and a presumed pathogenic V774M mutation, respectively. The most common mutations were identified including EGFRvIII and a presumed pathogenic V774M mutation, respectively. The most common mutations were identified including EGFRvIII and a presumed pathogenic V774M mutation, respectively.

Conclusion: Overexpression of molecular biomarkers of increased angiogenesis, EGFR and immune checkpoints provides novel targeted therapy options in malignant phyllodes tumors of the breast.

Methods

Thirty-six malignant PTs (including 8 metastatic tumors with two cases having matched primary and metastatic tumors) were profiled using gene sequencing (NGS and Sanger), gene copy number analysis, whole genome expression, and protein expression (immunohistochemistry).

Results (updated)

Phyllodes tumors (PT) of the breast are rare, fibro-epithelial neoplasms, constituting ≤1% of all breast cancers and are a therapeutic challenge as no effective targeted therapy has been reported yet. In the present study, we comprehensively profiled a series of malignant PTs of the breast in an attempt to potentially targetable pathways/biomarkers.

Figure 1. A-D. Primary malignant PT of the breast (A-B: H&E stain, 10-20x magnification) with a strong membranous EGFR protein overexpression (C: IHC stain) accompanied by EGFR gene amplification (D: dCISH).

Figure 2. A case of metastatic PT to the lung with peripheral PO-LI expression adjacent to the inflammatory cells and normal lung parenchyma; of note this case harbored RB1 gene mutation in both primary and metastatic tumor (case# 17).

Table 1. Results of multiplatform molecular profiling of 36 malignant PT cases.

<table>
<thead>
<tr>
<th>Case (PT)</th>
<th>PO-LI (PO)</th>
<th>EGFR (IHC)</th>
<th>EGFR (NGS)</th>
<th>EGFR (Sanger)</th>
<th>PIK3CA (microarray)</th>
<th>PTEN (IHC)</th>
<th>NUT (IHC)</th>
<th>NUT (Sanger)</th>
<th>TP53 (IHC)</th>
<th>TP53 (Sanger)</th>
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<tbody>
<tr>
<td>PT1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT2</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>PT3</td>
<td>+</td>
<td>+</td>
<td>-</td>
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Figure 3. Gene expression signature of six PT cases along with the normal breast tissue. In the heatmap, row represents genes and columns represent samples. Upregulated (red) is defined as a transcript with expression level that is >2x relative to normal breast control and down-regulated (blue) is defined as a transcript with expression level that is <2x relative to control. The expression of the normal breast is shown in the far left column.

Figure 4. Bar plots for 6 angiogenesis biomarkers differentially regulated in PT cases compared to normal breast tissue. The height represents the ratio of expression for the gene in the phyllodes case over the expression in the normal breast. For CYP3A5, three phyllodes cases had no detectable expression of the transcript and the values are depicted as 0 ratio. Normal breast expression ratio is set to ‘1’ for all 6 biomarkers and is depicted as the ‘black’ bar for all 6 genes.

Conclusions

This study provides additional support for comprehensive profiling in PT, which can identify several potentially targetable pathways including EGFR, angiogenesis, and immunotherapy for patients with locally advanced or metastatic tumors. Further prospective studies should confirm the clinical relevance of profiling-identified biomarkers.