MRI evaluation of breast cancer response post neoadjuvant treatment: correlation with miRNAs as biomarkers

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Aims and objectives

Breast cancer is the most common cancer in women worldwide. Neoadjuvant chemotherapy (NAC) has become increasingly used in the management of breast cancer. Initially introduced to facilitate resection of inoperable cancers, it is now also employed in early operable (stage T2 and T3) tumours, permitting less radical surgery [1-3]. Standard NAC regimens involve six to eight cycles (fortnightly) of combination chemotherapy regimens, commonly adriamycin and cyclophosphamide followed by docetaxel or paclitaxel, with the addition of trastuzumab for HER2/neu overexpressors [1,4]. However, response to treatment is not uniform; some patients respond better than others, with the range of response reported to be as varied as 4 to 34% with response rates as high as 50% in HER2/neu over-expressing subsets [4-6]. Magnetic resonance imaging (MRI) performed pre and post NAC in breast cancer evaluates therapeutic response preoperatively, being superior to other imaging modalities, namely mammography and ultrasound [7-9]. The gold standard for evaluating chemotherapeutic response is postoperative pathology.

In recent decades there has been a surge of research into the identification of circulating biomarkers for breast cancer, to facilitate early diagnosis, predict response to treatment and provide details of a tumour’s molecular blueprint to facilitate prognostication. Mi(cro)RNAs are a class of small, non-coding RNA molecules that play important roles in almost all biological processes by regulating gene expression at the post-transcriptional level, playing a functional role in both physiological and pathological states. They are intricately involved in tumorigenesis and so the ability to identify specific miRNAs as diagnostic, prognostic, predictive and therapeutic targets has become a focus of research worldwide. MiRNA expression profiles can classify tumours by type or clinico-pathological characteristics, which has potential utility in cancer diagnostic, prognostic and predictive settings [10, 11]. MiRNAs are detectable in blood and have promising biomarker potential. MiR-195 has been shown to be overexpressed in the blood of women with breast cancer compared to that of controls and is of interest to our group [12].

Little is known about the relationship between miRNA expression in breast cancer and imaging features (radiogenomic features). NAC provides an excellent platform to evaluate the link between miRNA expression and MR imaging features. Tumour response can be evaluated with the tumour in situ. This study utilised the setting of NAC to explore the relationship between MRI response, pathological response and miRNA expression.
Methods and materials

Material and Methods

Ethical approval was granted by the Clinical Research Ethics Committee, Galway University Hospital.

Patient Selection

11 Patients who underwent NAC in Galway University Hospital in 2012 were invited to participate in this prospective cohort study, once they were over 18 years of age and provided written informed consent.

Breast MRI

Breast MRI was performed (Magnetom Espree 1.5 Tesla, Siemens Medical Systems, Erlangen, Germany) pre and post-NAC. The patient lay in the prone position with the use of a dedicated 8-channel breast surface coil (Siemens Medical Systems). An intravenous cannula was sited in advance and gadolinium-containing contrast agent was administered during the study. Sequences were acquired according to departmental standard bilateral breast imaging protocol. Sagittal T2weighted and sagittal T1weighted sequences were acquired. Six series of sagittal T1 weighted fat saturated sequences were acquired, the first series was non-contrast enhanced, during the second there was a bolus injection of gadolinium DTPA (Gadovist 1.0 mmol/mL) with a total dose of 0.1ml per kilogram body weight, which was immediately followed by a 20ml saline flush. The peak enhancement was in the third series and the washout in the sixth series. An axial T1 weighted fat saturated post contrast sequence was then acquired. Post processing included subtraction for rapid wash-in and rapid wash-out and maximum intensity projection (MIP).

Breast MRI was performed before commencing NAC, to establish baseline, and upon completion of NAC to assess residual disease (Figure 1). Response to treatment was evaluated using the RECIST criteria [13]. Complete response (CR) denotes complete radiological resolution of the tumour. Partial response (PR) is defined as a decrease of the sum of the longest individual lesion axes by more than 30%. The term progressive disease is defined as an increase of the sum of the longest axes of individual lesions by more than 25%. The remainder are classified as 'stable disease' (SD).

Blood Sampling
One 4-8ml bottle of whole blood (EDTA) was taken from each patient at the designated time-points outlined in Figure 1 (diagnosis, peri-NAC and post-NAC). Whole blood samples were stored at 4°C until transfer to the laboratory in the Discipline of Surgery at National University of Ireland, Galway. Once received in the laboratory, blood samples were again refrigerated until processing.

**RNA extraction**

Total RNA was extracted from 1ml of blood using TRI Reagent BD (Molecular Research Centre, Inc) [14]. RNA concentration and integrity were analysed by NanoDrop spectrophotometry (NanoDrop ND-1000 Technologies Inc., DE, USA) and Agilent Bioanalyzer RNA 6000 NanoChip Kit Series II (Agilent Technologies, Germany), respectively.

**MiRNA expression by RQ-PCR**

Expression of the target miRNA (miR-195) and endogenous controls (miR-425 and miR-16) was determined by RQ-PCR using TaqMan miRNA assays (Applied Biosystems). After RNA extraction, 100ng total RNA was reverse transcribed using stem-loop primers and Multiscribe reverse transcriptase. Triplicate PCR reactions in final volumes of 10mls were performed on 96 well plates, each containing an interassay control (IAC). Standard thermal cycling conditions were employed on a 7900 HT instrument (Applied Biosystems). Raw fluorescence (cycle threshold, C\text{T}) data were calculated. Low C\text{T} values indicated high miRNA expression, and vice versa. Threshold standard deviation for inra- and interassay replicates was 0.28. PCR amplification efficiencies (E) were determined for each miRNA and Taqman miRNA assay using the formula $E = (10^{21/\text{slope21}})6100$, using the slope of the semi-log regression plot of C\text{T} versus log input of cDNA (10-fold dilution series of five points). A threshold of 10% above or below 100% was accepted. C\text{T} values were scaled to the lowest expressing sample and normalized to miR-16 and miR-425, which have been shown to be stably expressed in breast cancer and an appropriate endogenous control [15]. MiRNA expression was calculated by the comparative cycle threshold, using qBasePLUS software (Biogazelle, NC, Belgium).

**Histology**

All patients had pre-NAC core needle biopsy of the primary breast tumour, under ultrasound guidance (Philips ATL HDI 5000, Philips Healthcare, Andover, AM). Ultrasound guided core biopsies were obtained following administration of local anaesthetic (2% lidocaine with adrenaline, Braun Medical, Pennsylvania, USA) using a 14G core biopsy needle (14G Achieve™, Carefusion, Illinois, USA) and a high frequency (7.5 Hz or 12 Hz) linear array transducer (Philips Healthcare). Both the pre NAC core biopsy and the post-NAC surgical resection specimen were reviewed in a standard
manner by a consultant histopathologist and discussed at the multidisciplinary meeting (MDM).

**Statistical Analysis**

Statistical analysis was performed using Minitab V17 (Minitab Ltd., Coventry, UK). A log transformation ($\log_{10}$) was performed when data was not normally distributed. A p-value of less than 0.05 was deemed to be significant.
Fig. 1: Breast MRI was performed at diagnosis prior to commencing NAC and upon completion of NAC prior to surgery. Three blood samples were obtained for each patient prior to surgery; One at initial diagnosis, peri-NAC (before cycle 4 if undergoing a 6 cycle regimen, before cycle 5 if undergoing an 8 cycle regimen) and the third upon completion of NAC prior to surgery.

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Results

Patient details are presented in Table 1 (Figure 2). The mean patient age was 50.3(±8.3) years. There was no correlation between MRI response and pathological response in this patient cohort (p=0.555). MRI responses varied. MRI complete response was achieved in 2 patients (partial n=7, stable n=1, progression n=1). There was no significant difference between MRI response groups in circulating \textit{miR-195} expression at diagnosis (p=0.478, see Figure 3), peri-NAC (p=0.745) and post-NAC (p=0.689, see Figure 4). There was no significant difference between pathological response groups in circulating miR-195 expression at diagnosis (p=0.259), peri-NAC (p=0.515) and post-NAC (p=0.502).
Fig. 1: Breast MRI was performed at diagnosis prior to commencing NAC and upon completion of NAC prior to surgery. Three blood samples were obtained for each patient prior to surgery; One at initial diagnosis, peri-NAC (before cycle 4 if undergoing a 6 cycle regimen, before cycle 5 if undergoing an 8 cycle regimen) and the third upon completion of NAC prior to surgery.

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### Table 1: Patient Details

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<table>
<thead>
<tr>
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<tr>
<td>Mean patient age (±st. Dev)</td>
<td>50.3 (± 8.3) years</td>
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<tr>
<td>Mean tumour size at MRI on diagnosis (±st. Dev)</td>
<td>4.2 (± 1.25) cm</td>
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<tr>
<td>Mean tumour size at MRI post NAC (±st. Dev)</td>
<td>3.1 (± 1.56) cm</td>
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**MRI Response**

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<td>Partial</td>
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<tr>
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**Pathological Reponse**

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<tr>
<td>Partial</td>
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<tr>
<td>Poor/Progression</td>
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</table>

**Fig. 2:** Overview of patient and breast tumour characteristics.

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Fig. 3: There was no significant difference between MRI response groups in circulating miR-195 expression at diagnosis (p=0.478).

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Fig. 4: There was no significant difference between MRI response groups in circulating miR-195 expression post-NAC (p=0.689). Data for the stable response group is missing (n=1).

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Conclusion

MRI is performed pre and post-NAC to help evaluate response to NAC preoperatively. MiR-195 is not a useful substitute for MRI for evaluation of response in this cohort. However, this preliminary study contributes towards the evolving field of radiogenomics. The current era of tailored treatment has a need to identify biomarkers to facilitate the correlation of radiological findings with those on a molecular level with the aim of enhancing the application of non-invasive imaging modalities in breast cancer.
Personal information

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