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Detection of some microRNAs expressions in tissues of British Women diagnosed with Breast cancer

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Abstract:

MicroRNAs (miRNAs) are small, non coding RNA, found to play critical roles in tumor progression. The aim of this project was to investigate the expression of miR-21, miR-26b, miR-429 and miR-378 in human breast cancer tissues. By using TaqMan qRT-PCR assay to detect the expression of these selected miRNAs in 12 pairs of breast cancer tissues and their corresponding noncancerous breast tissues. Data indicated that the relative level of miR-21 was up regulated in cancer tissues (p 0.0014), miR-26b was significantly down regulated in breast cancer tissues (p 0.0017), While the novel expression of miR-429 was significantly up regulated in breast cancer tissues (p 0.0131) and novel down expression of miR-378 in breast cancer tissues (p 0.0006). These microRNAs might be a potential molecular biomarker for early detection of breast cancer in women.

Keyword: *MicroRNA, expression, Breast cancer, Tissues real time PCR.*

Introduction:

Breast cancer represents one of the most commonly diagnosed cancers among women, accounting for about 30% of patients [1]. Early screening for breast cancer allows early stage diagnosis of the malignancy and reduces mortality. Despite the dedication of research and development of new biomarkers for diagnosis and prognosis, unpredictable response and development of resistance to adjuvant therapy remain as major challenges in breast cancer management [2].

The emergence of small non-protein-coding RNAs, small (20–24 nucleotides), that post-transcriptionally modulate gene expression and playing important roles in oncogenesis, opened new opportunities for early cancer diagnosis [3,4]. MicroRNAs play diverse roles in tumorigenesis and in the progression of breast cancer, and may act as oncogenes, tumor suppressors and modulators of tumor proliferation, invasion, apoptosis and therapy resistance [5,6,7].

Several techniques have been used such as bead-based flow cytometric for miRNA expression profiling method; they identified 133 miRNAs expressed in human breast tumors, which could be used to classify breast cancer into prognostic subtypes [8]. Another study mentioned that five of miRNAs (miR-421, miR-486, miR-503, miR-720 and miR-1303) to be predictive for inflammatory breast cancer with an overall accuracy of 89% [9]. These data clearly indicate that specific miRNA expression patterns are associated with the biological and clinical properties of human breast cancer.

In the present study, qRT-PCR assay was performed to detect the expression of miR-21, miR-26b, miR-429 and miR-378 in breast cancer and corresponding noncancerous breast tissues. Our data showed that miR-497 was significantly down-regulated in BC tissues and could be served as a potential molecular biomarker for the prediction of poor prognosis

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Materials and Methods:

• Specimens

In this study, 12 paired breast cancer and non-cancerous specimens were collected from the british women undergo surgery and then tissues frozen at -80˚C in LHS lab/ Aston University until extraction time. All cancer tissue samples were confirmed as invasive, ductal breast cancer by pathologists.

• Extraction of microRNAs from tissue samples

MicroRNAs were extracted from tissue samples using microRNA extraction kit (InvetrogenTM / life technologies/ USA) using 5 mg of tissue specimens. Then, 300µl of binding buffer mixed with tissue and homogenized in a procedure described in the kit protocol. The microRNA at the end was eluted by 50-100 µl of nuclease free water. The microRNA concentration was measured using NanoDrop-1000 spectrophotometer (Nano Drop Technologies, USA).

• Perform cDNA and real-time PCR using TaqMan

The extracted microRNA was reversed transcribed using TaqMan microRNA reverse transcription kit (ApplidBiosystems, USA) according to the manufactures protocol. Then 5 µl of microRNA was reversed transcribed in a 15 µl reaction volume for each assay. TaqMan microRNA probes were used (miR-21, let-7a, miR-222, miR-26b, miR-27a, miR-15b, miR-34a, miR-34b, miR-205, miR-218, miR-378, miR-429 and miR-191 was used as an endogenous control) were used to quantify microRNA in real time PCR assays according to manufactures protocol. Real time PCR assays were performed in a 20 µl reaction volume using Stratagen 3000p real time system.

• Statistical analysis

The relative quantitative gene expression level was evaluated using the ΔΔCt comparative Ct method [10]. Fold inductions were calculated using the formula 2^ (ΔΔCt) where:
ΔCt=Ct (target gene)-Ct (reference gene)
ΔΔCt=ΔCt (treated) − ΔCt (control).
Student t test p value was calculated using GraphPad Prism 6.

Results:

TaqMan qRT-PCR assay was performed to detect the expression of miR-21, miR-26b, miR-429 and miR-378 in 12 pairs of breast cancer and corresponding noncancerous breast tissues. The expression of miR-21 was up regulated in cancer tissues as shown in figure 1 (A), with p value (0.0014). MiR-26b was significantly down regulated in breast cancer tissues with p value (0.0017) as in figure 1 (B). While in figure 1 (C), the novel expression of miR-429 was significantly up regulated in breast cancer tissues and p value ranged (0.0131). The novel expression of miR-378 was shown in figure 1 (D), down expression of miR-378 in breast cancer tissues with p value (0.0006).

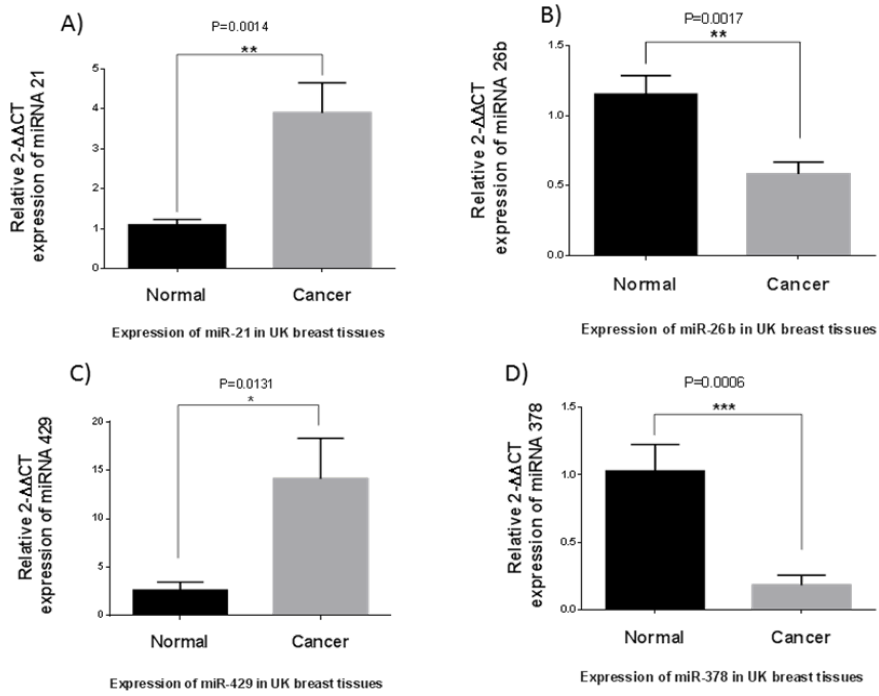

Figure 1: TaqMan qRT-PCR detection of relative microRNAs expression in breast cancer tissue samples compared to its matched non-cancerous tissues. **A)** The expression level of miR-21 in breast cancer tissues was significantly higher than that in corresponding non-cancerous breast tissues (p=0.0014). **B)** The expression level of miR-26b in breast cancer tissues was significantly lower than that in corresponding non-cancerous breast tissues (p=0.0017). **C)** The novel expression level of miR-429 in breast cancer tissues was significantly higher than that in corresponding non-cancerous breast tissues (p=0.0131). **D)** The novel expression level of miR-378 in breast cancer tissues was significantly lower than that in corresponding non-cancerous breast tissues (p=0.0006). MicroRNA-191 was used as an endogenous control. Corresponding p values analyzed by t-test are indicated.

Discussion:

Breast cancer represents a group of heterogeneous diseases that show various biological and clinical characteristics [11]. Patient management is currently based on easily identifiable clinical and pathological characteristics, which only partially reflect disease heterogeneity. Many principal factors, such as patient age, status of axillar lymph nodes, tumor size, histological traits, status of hormonal receptors and HER2, have been used for the prediction of the prognosis of breast cancer patients for many years [12,13], but their roles in determining the individual risk level of the patient are quite limited. Therefore, it is still needed to exploit clinically useful, readily available prognostic markers in the management of BC.

MicroRNAs, important regulators of mRNA and protein expression, are emerging as important modulators of essential biological functions, including cellular development, apoptosis, metabolism and oncogenesis [14]. They represent a novel biological entity with potential value as tumour biomarkers, which can improve diagnosis, prognosis, and monitoring of treatment response for human cancers [15]. Mertens-Talcott et al. reported that miR-27a expression was associated with poor overall survival in patients with breast cancer, suggesting that miR-27a could be a valuable marker of breast cancer progression [16]. Over-expression of miR-21 was determined in tissues of breast cancer and the possibility the use of this miRNA to discriminate between breast cancer and non- tumor tissues with high specificity and sensitivity [17]. Bojian and Haorong, 2012 found that low expression of miR-378 inhibit cell proliferation, cell cycle progression, cell migration as well as invasion and they found that miR-378 act as tumor suppressor in gastric cancer suggesting its novel expression useful in diagnostic and therapeutic role in cancers [18]. Down regulation of miR-26b has been confirmed in breast cancer and caused fibroblast migration and invasion to increase three folds [19]. MicroRNA-429 expression may modulate the tumorigenesis in gastric cancer and shown to play an oncogenic [20]. Li and coworker (2013) found that high expression of miR-429 has been detected in colorectal cancer tissues than in non tumor tissues [21].

Thus, the aim of this study was to determine the expression of miR-21, miR-26b, miR-378 and miR-429 in tissues of breast cancer and its matched normal tissues. Here, the expression of miR-21, miR-26b, miR-378 and miR-429 were detected in 12 pairs of breast cancer and corresponding non-cancerous breast tissues, and showed that the relative expression level the relative level of miR-21 was up regulated in cancer tissues (p 0.0014), miR-26b was significantly down regulated in breast cancer tissues (p 0.0017), While the novel expression of miR-429 was significantly up regulated in breast cancer tissues (p 0.0131) and novel down expression of miR-378 in breast cancer tissues (p 0.0006) compared to non cancerous normal matched tissues. MiRNA-21 is over-expressed in breast cancer tissues on cell invasion may be due to the regulation of metalloproteinase 3 (TIMP3) expressions [22]. Down regulation of miR-26b R-26b suppressed the TNFα- and doxorubicin-activated NF-κB signaling in HCC cells, and dramatically sensitized cancer cells to the doxorubicin-induced apoptosis [23]. Low expression of miR-378 was detected in the study of (Deng et al., 2013) in gastric cancer tissues. The low expression in tissues was related to the presence of CpG island methylation on miR-378, this study support the idea that miR-378 has tumor suppressor properties [24]. The dual function of miR-429 has been detected in two different studies as an oncogenic in colorectal cancer and as tumor suppressor in gastric cancer, and suggested the differences due to difference of cellular context or alternatively the targeted genes [25].

The result indicated that significant expression of miR-21, miR-26b, miR-378 and miR-429 in tissues of breast cancer might be an important early biomarker for breast cancer detection.

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الكشف عن تعبير بعض الـ microRNAs في انسجة نساء بريطانيات مصابات بسرطان الثدي

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الخلاصه:

جزيئات microRNAs هي صغيرة الحجم, RNA غير مشفر, تلعب دور مثالي في نمو الورم. هدفت الدراسة الحالية لتحديد تعبير miR-21, miR-26b, miR-429 و miR-378 في انسجة سرطان الثدي. وبأستخدام تقنية TaqMan qRT-PCR للكشف عن تعبير هذه الجزيئات المختارة في 12 زوج من انسجة سرطان الثدي ومقابلاتها من انسجة الثدي غير السرطانية الطبيعية. اظهرت النتائج ان مستوى تعبير miR-21 كان مرتفعا في انسجة الثدي السرطانية (p 0.0014), miR-26b كان منخفضا انخفاضا معنويا في انسجة الثدي السرطانية (p 0.0017), بينما التعبير المثالي الجديد للـ miR-429 كان مرتفعا معنويا في انسجة الثدي السرطانية (p 0.0131) و انخفاض في تعبير miR-378 المثالي الجديد في اسجة سرطان الثدي (p 0.0006). خلصت هذه الدراسة الى امكانية استخدام ال microRNAs المستخدمة كمؤشرات وراثية اولية لحدوث سرطان الثدي عند النساء.