Investigating the Relationship between Cadherin Expression and Invasiveness in Breast Cancer

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Introduction

Cadherins represent a large superfamily of transmembrane receptors, which allow cells to interact with other cells and facilitate cell signaling. Cadherins can be classified into three types. Types I, II and III cadherins are all expressed in the mammary gland. However, although the role of the type I cadherins in cancer has been extensively studied, very little is known about the potential role type II cadherins might play in this disease [2].

The two main type II cadherins known to be expressed in breast tissue are cadherin-5 and cadherin-11 [1]. Cadherin-5, also known as vascular endothelial cadherin, is normally involved in regulating the morphology and stability of blood vessels [3]. In invasive breast cancers the regulation of cadherin-5 is altered such that it becomes expressed in mammary epithelial and endothelial cells, which enhances the ability of these two cell types to adhere together [1]. Cadherin-11, or OB-cadherin, is also aberrantly expressed in breast cancer and may promote migration of the cancer to bone [3].

The type I cadherins, Cadherin-3 and cadherin-4, are also expressed in breast cancer cell lines [1]. Cadherin-3 (P-cadherin) is overexpressed in invasive breast cancer and may enhance the migration of breast cancer cells [4]. Not much is known about the function of cadherin-4 (R-cadherin) but in breast cancer, cadherin-4 expression is repressed, giving cells a metastatic phenotype [1].

The aim of this study was to determine the cadherin expression levels in four different breast cancer cell lines, MDA-MB-231, SK-BR-3, T47D and MCF7. These cell lines represent four different types of breast cancer, and once we determine the level of cadherin expression in each line, we can study how cadherin expression relates to invasiveness in different breast cancer cell types.

Results

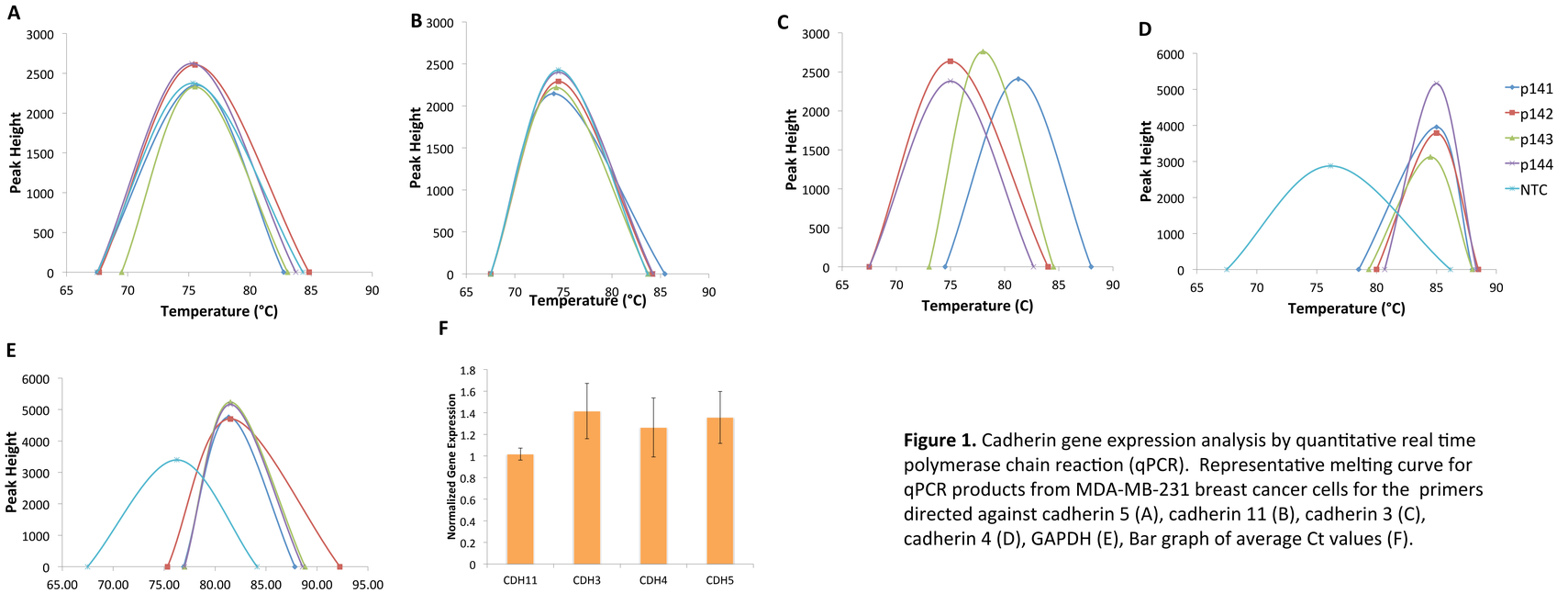

Figure 1. Cadherin gene expression analysis by quantitative real time polymerase chain reaction (qPCR). Representative melting curve for qPCR products from MDA-MB-231 breast cancer cells for the primers directed against cadherin 5 (A), cadherin 11 (B), cadherin 3 (C), cadherin 4 (D), GAPDH (E), Bar graph of average Ct values (F).

Materials and Methods

Cell Culture: All cell lines were cultured and grown in DMEM-F12 media plus 10% FBS, 1% sodium pyruvate, 1% PSG.
Extraction of RNA: The cells were cultured in a 6 well plate and RNA was extracted using TRIZOL Reagent according to the manufacturers instructions. The yield and concentration of RNA was then quantified using the Biotek spectrophotometer.
RT-PCR and qPCR: RT-PCR was performed using 1.0 ug of RNA from each cell line. The Verso cDNA kit was used. For qPCR the following primers were used: Cadherin 5, Cadherin 11, Cadherin 3, Cadherin 4, GAPDH, and Ribosomal Protein 18. The primers were diluted to a 1M concentration and then combined with SYBR Green. This mixture was then added to a 96 well plate (7.5 mL). The cDNA from RT-PCR was diluted, and 2.5 mL was added into each well of the 96 well plate. The following cycling parameters were used for PCR: 3 minutes at 95°C, 95°C for 10 s, 55°C for 10 s, and 72°C for 30 s, 39 cycles. Then 95°C for 10 s, and 1 degree intervals from 65C to 95°C for 5 s each.
Gel Eletrophoresis: I prepared a 2% agarose gel. I prepared the samples by adding loading dye to the sample in a 1: 10 ratio. I loaded 10 uL of mixture into each well with one well containing DirectLoad Wide Range DNA Marker (Sigma Aldrich). Once all the samples were loaded I ran the gel at 100V for 30 to 40 minutes. I then viewed the gel using the FluorChem E System (Protein Simple)

Conclusions

	Genes Expressed				
Cell Line	**Cadherin 3**	**Cadherin 4**	**Cadherin 5**	**Cadherin 11**	**GAPDH**
MDA-MB-231	-	+	-	-	+
SK-BR-3	+	-	-	-	+
T47D	-	-	-	-	+
MCF7	-	-	-	-	+

Future Goals

Future goals would be to use cells that have been shown to express these cadherins as a positive control. Also another goal would be to relate the expression level of the cadherins with the invasiveness of the cell lines studied.

References

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