# MUPP1 Expression in Breast Cancer Cells

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## Introduction

Breast cancer usually begins in the lobules, where milk is produced, or in the ducts, which transfer the milk from the lobules. Breast cancer metastasis occurs when a cancer cell leaves its tissue of origin, namely, the breast tissue, and migrates to a different tissue type. Metastasis is possible when the integrity of the structures holding the cells in place is compromised.

One of these types of structures are the tight junctions. Tight junctions are a collection of proteins that seal adjacent cells together at the apical surface. Tight junctions hold the cells together as well as restrict movement of substances between cells (Hamazaki et al. 2002). In metastasis, tight junction function is disturbed so the metastatic cell is able to escape its tissue of origin (Musch et al. 2006, Martin and Wiang 2008). MUPP1 has been known to interact with tight junction proteins such as cadherins (Jeansonne et al. 2003, Lanaspa et al. 2007). MUPP1 is a scaffolding protein at the tight junctions and it also the anchors tight junctions to F-actin (Lanaspa et al. 2007). Actin also promotes cell to cell adhesion through its interaction with cadherins (Hall 2009).

MUPP1 levels are known to decrease in patients with metastatic breast cancer (Martin et al. 2004). MUPP1 is a 7454 base pair gene that codes for 2054 amino acids which contain 13 PDZ domains. A PDZ domain is a structure of about 70 to 90 amino acids that are characterized by the formation of 6 beta-helices and 2 alpha-helices in a specific orientation (Ranganathan and Ross 1997). The structure of PDZ domains allow them to interact with cytoplasmic proteins as well as anchor to the cytoskeleton (Banerjee and Wedegaertner 2004).

The purpose of this study was to determine MUPP1 expression and alternative splicing in various breast ductal carcinoma epithelial cell lines.

# Methods

RNA was purified from MDA-MB-231 and HeLa cells by using the QIAGEN RNeasy Plus Mini Kit and following the protocol from the 2010 QIAGEN RNeasy Plus Mini Handbook: Purification of Total RNA from Animal Cells.

RNA used in this experiment was from the MDA-MB-231, MDA-MB-435S, MCF-7, MDA-MB-468, TD-47, and Hela cell lines. RNA from the MDA-MB-231 cell line was used twice in each experiment in this project. One of the MDA-MB-231 RNAs used was newly purified and the other was from previously purified cells. The newly purified MDA-MB-231 RNA is shown in the first lane in each of the agarose gel figures (Figures 1 and 3). The RNA from previously purified MDA-MB-231 cells is shown in the sixth lane in each of the agarose gel figures (Figures 1 and 3).

In this project, RT-PCR was done using the QIAGEN OneStep RT-PCR Kit and following the QIAGEN OneStep RT-PCR Kit Handbook where 5 ug/ul RNA solutions were used.

RT-PCR was done to amplify specific regions near the beginning (exon 2 to 3), middle (exon 19 to 20) and end (exon 43 to 44) of the MUPP1 gene. A region in the GAPDH gene was also amplified. All primers are written in the 5` to 3` direction.

The forward primer amplifying a region from exon 2 to 3 was GAGAACGTGGGGATGTAGCA and the reverse primer was GGCTGAGATGAGGAACG.

The forward primer amplifying a region from exon 19 to 20 was

GCTCTGAGTACCTGCTTGAA and the reverse primer was CATCTCGACTAATGGCACCT.

The forward primer amplifying a region from exon 43 to 44 was GAGGAGACGTGAGTGTGGTC and the reverse primer was AGCCTAAGCCATCTGGTCCT.

The forward primer for GAPDH was TGGGTGTGAACCATGAGAAG and the reverse primer was TGTCGCTGTTGAAGTCAGAG.

RT-PCR using these primers was done three times, twice at 40 cycles and once at 35 cycles. The thermal cycler was set up as follows: 50°C for 30 minutes, 95°C for 15 minutes and then the cycle parameters were: 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. After the cycles, the temperature was set at 72°C for 2 minutes and then held at 4°C. The amplified products were run on a 1% agarose gel and visualized using a BioRad UV Transilluminator. One RT-PCR product from each primer pair was purified using the 2008 QIAquick Spin Handbook: QIAquick PCR purification K Protocol using a microcentrifuge. The purified products were then to GENEWIZ for sequencing.

Primers were designed to amplify exon 18, exon 35 and exons 26 and 27.

To amplify exon 18, primers were made in exons 17 and 19. The forward primer was TGATCCAGCAAGCACTGTG and the reverse primer was CACCACAAGAACTGCCATG.

To amplify exon 35, primers were made in exons 34 and 36. The forward primer was AGATGCCGATGGAAGAC and the reverse primer was CAGTGACTCAGATGTACTGGATC.

To amplify exons 26 and 27, primers were made in exons 24 and 28. The forward primer was TCGGCTAAGCAATGGAG and the reverse primer was GCAGATGACTGTGTGTGA.

RT-PCR using these primers was done twice, once with an elongation time of 30 seconds and the second time with an elongation time of 60 seconds. The thermal cycler was set up as follows: 50°C for 30 minutes, 95°C for 15 minutes and the 40 cycle parameters were: 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 or 60 seconds. After the cycles the temperature was set at 72°C for 2 minutes and then held at 4°C. The amplified products were run on a 1% agarose gel and visualized using a BioRad UV Transilluminator. The products from the MDA-MB-468 cell line's RNA were gel purified and sent to GENEWIZ for sequencing.

# Results

There is no clear differential expression of MUPP1 between the MDA-MB-231, MDA-MB-435S, MCF-7, MDA-MB-468, TD-47, and the HeLa cell lines (Figure 1). All the cell lines show the same band intensity in each primer pair, indicating that the part of gene amplified does not differ in expression levels between the cell lines.

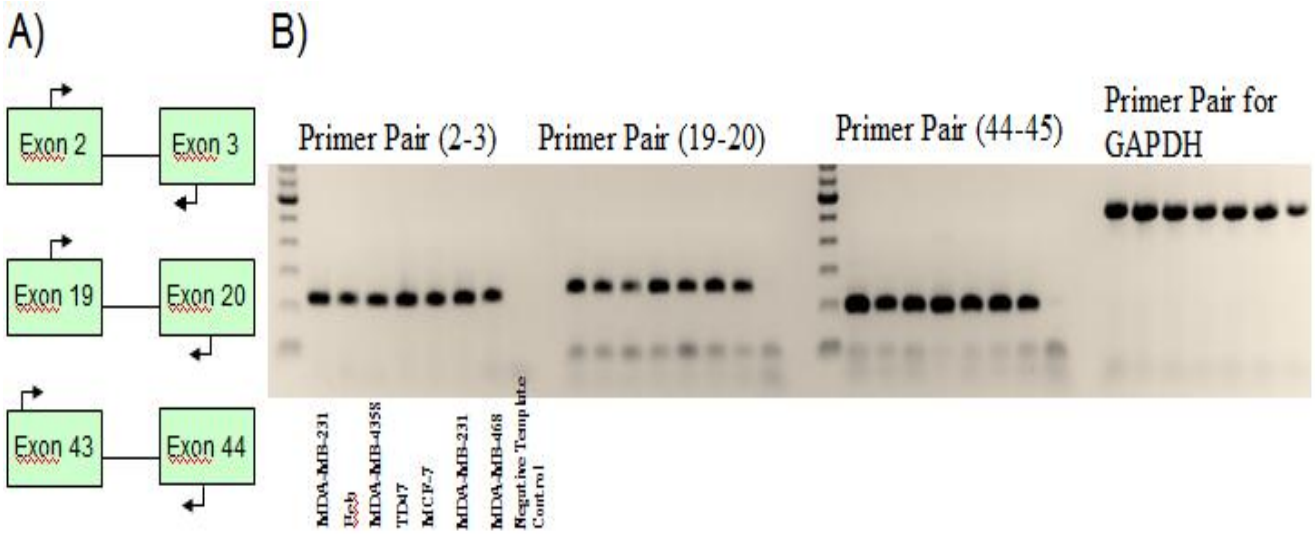


Figure 1: A) A depiction of the primer placements in the MUPP1 transcript. The arrows represent the direction and approximate location of each primer. B) Gel electrophoresis of the RT-PCR products after 35 cycles are visualized on a 1% agarose gel. Each set of bands shows

the amplified product from one primer pair in each cell line. The GAPDH gene was used as a positive control.

Some transcripts of the MUPP1 gene are missing various exons (Figure 2). Primers were designed to amplify regions of alternatively spliced exons to see if these variations existed in the cancer cell lines tested.

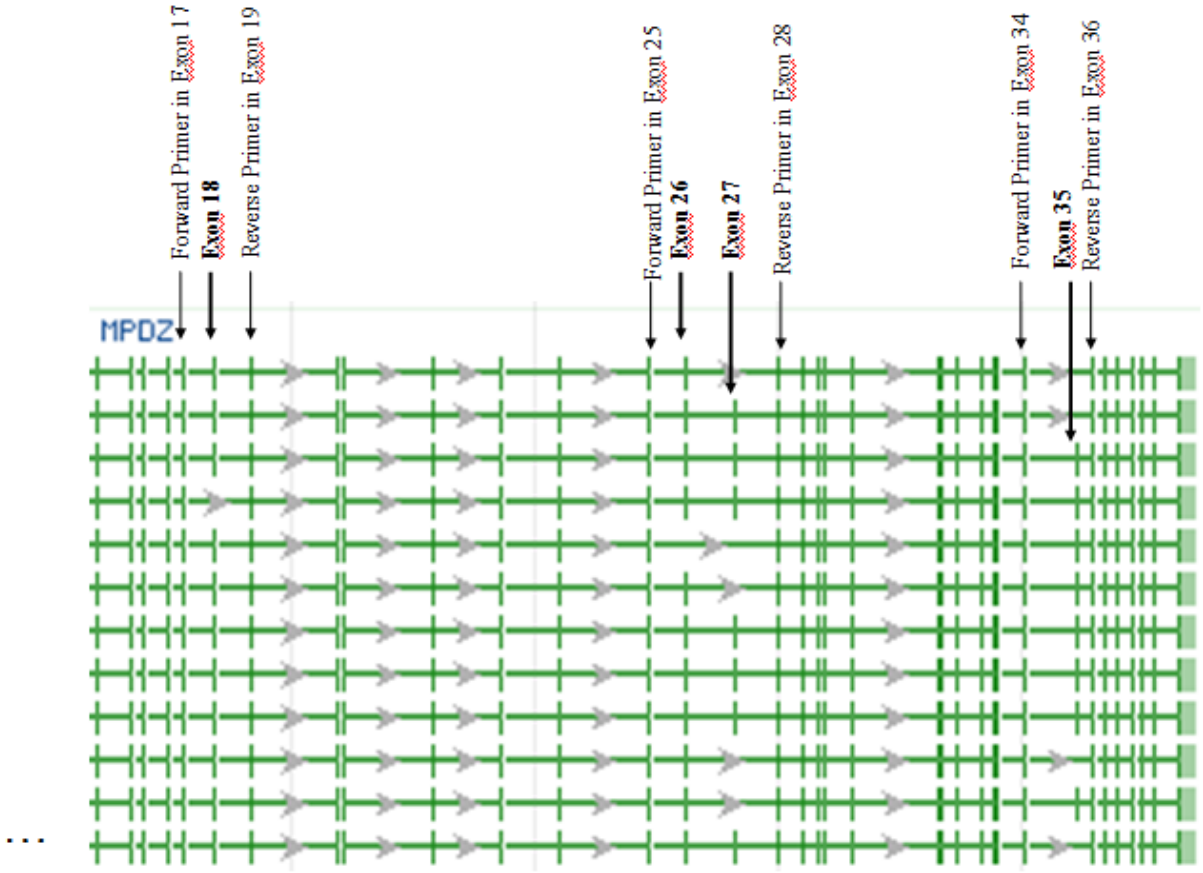


Figure 2: The C-terminal end of MUPP1's gene table from the NCBI website. Each horizontal line represents one variation of MUPP1's transcript. The vertical lines represent exons and the horizontal lines in between them symbolize introns. Primers were designed to amplify the exons missing in some transcripts.

The cancer cell lines showed differential splicing using the primer pairs in Figure 2 (Figure 3). The MDA-MB-435S, MCF-7, and MDA-MB-468 cell lines have a darker band of product B as compared to the other cell lines, while product A has a similarly dark band in all

the cell lines. This indicates that the MDA-MB-435S, MCF-7, and MDA-MB-468 cells lines have more product with exon 18 spliced out as compared to the other cell lines. All of the cancer cell lines have a darker band for product C as compared to product D, which indicates that there is a tendency of exon 35 to not be spliced out in these cell lines.

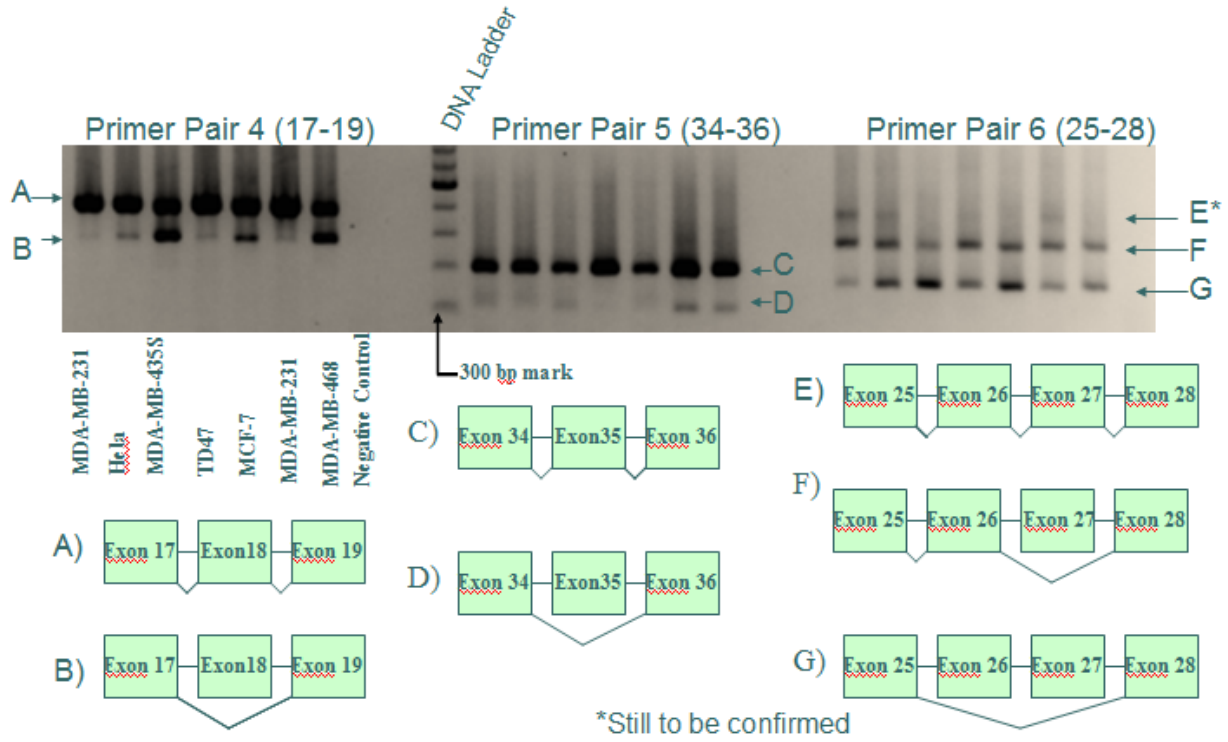


Figure 3. Gel electrophoresis of the RT-PCR products from the primers in Figure 2 on a 1% agarose gel. Each band corresponds to a possible product depicted below the gel.

Since there are variations in the MUPP1 transcript, the different transcripts would code for different sized proteins. This is illustrated in a western blot done by Abcam (Figure 4). In this western blot, the antibody is made to a conserved region in MUPP1. The antibody is made to amino acids 775-825 which are coded for by exons 16 and 17. Exon 16 and 17 are not alternatively spliced out.

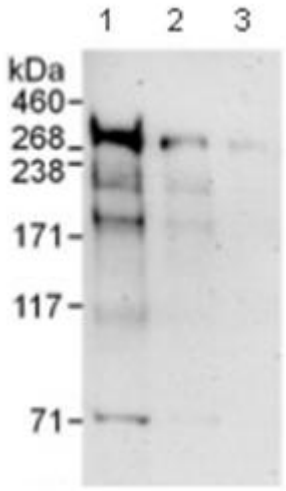


Figure 4. A western blot of MUPP1 done by Abcam using .01 ug/ml anti-MUPP1 antibody. There is 50 ug HeLa cell lysate in lane 1, 15 ug in lane 2, and 5 ug in lane 3. The blot shows different sized bands of MUPP1.

# Discussion

The MDA-MB-231, MDA-MB-435S, MCF-7, MDA-MB-468, TD-47, and the HeLa cell lines show no differential expression of the MUPP1 gene (Figure 1). However, there are spliced exons in every cell line. None of the spliced exons cause a frameshift in the transcript. Exon 18 is 117 base pairs and it codes for 39 amino acids. Exon 35 is 87 base pairs and it codes for 29 amino acids. Exon 26 is 111 base pairs and codes for 37 amino acids and exon 27 is 99 base pairs long and codes for 33 amino acids. When the MUPP1 gene is missing both exon 26 and 27 (Figure 3E), the protein is missing 70 amino acids. Even though some amino acids are missing in some transcripts, the amino acids coded for after the alternatively spliced exon are not changed.

Interestingly, none of the exons considered in Figures 2 and 3 coded for any of MUPP1's 13 PDZ domains. Therefore, these proteins still have the PDZ domains to potentially anchor to the cytoskeleton and interact with the tight junctions.

A western blot of MUPP1 shows different sized bands (Figure 4), which is expected since the transcript is alternatively spliced (Figure 3). An implication of the splicing is that protein recognizing compounds such as antibodies need to designed so they recognize an unspliced epitope on MUPP1.

Since none of splicing events caused an early stop codon or any other frameshift, it is likely that the amino acids coded for after the splicing events are important for MUPP1. Further protein analysis should determine the significance of differential splicing in the metastatic process.

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