2014

Cytoplasmic Polyadenylation Element Binding (CPEB) Protein 2 splice variants CPEB2A and CPEB2B affect the hypoxic response and triple-negative breast cancer metastasis

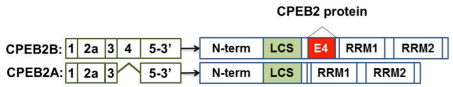
Ryan Johnson
Virginia Commonwealth University

Cytoplasmic Polyadenylation Element Binding (CPEB) Protein 2 splice variants CPEB2A and CPEB2B affect the hypoxic response and triple-negative breast cancer metastasis

Ryan M. Johnson, Charles E. Chalfant, Margaret A. Park
Department of Biochemistry, Cell and Molecular Biology; the Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia, USA.
Hunter Holmes McGuire VAMC, Richmond VA, USA.

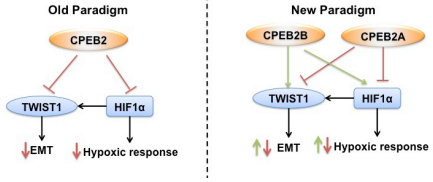
Triple-Negative Breast Cancer (TNBC)

- Tumors lacking estrogen receptor (ER), progesterone receptor (PR), and epithelial growth factor receptor 2 (HER2)
- Responsible for 15%-20% of all breast cancers
- Incredibly poor prognosis due to high metastatic rate
- Few clinical trials, lack of knowledge regarding an obscure cellular pathway



CPEB2: An Alternative Paradigm

- Cytoplasmic Polyadenylation Element Binding Protein 2 (CPEB2) has been known to be heavily involved with stress response mechanisms and assumed to properly regulate cell stress response.
- New findings exhibit alternatively spliced isoforms of the protein. CPEB2A is hypothesized to properly inhibit stress response in TNBC while CPEB2B (includes Exon 4) seems to be overexpressing stress response mechanisms.
- The stress response mechanism with HIF1-alpha and TWIST1 is shown to be a proponent in TNBC metastasis



Assays confirm difference in isoform ratio

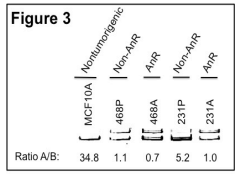


Figure 3: CPEB2A/B ratio dramatically decreases after anoikis resistance is acquired. MCF10A, MDA-MB-468 parental (468P), MDA-MB-468 AnR (468A), MDA-MB-231 parental (231P) and MDA-MB-231 AnR (231A) were plated onto poly-lysine-coated plates. RNA was harvested and first strand cDNA synthesis was performed. Competitive RT-PCR was then performed using primers corresponding to the region surrounding exon 4. Ratios were determined using ImageJ.

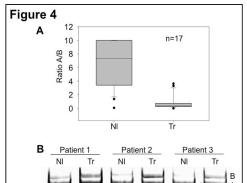


Figure 4: CPEB2 splicing is dysregulated in breast cancer patient samples. RNA was extracted from 17 tumor (Tr) and patient-matched normal tissue (NI) from breast cancer patients. First-strand cDNA synthesis was then performed and samples were subjected to qPCR using custom CPEB2A and B primer/probe sets (A) or competitive RT-PCR using primers designed to flank CPEB2 exon 4 (B). CPEB2A/B ratios were determined either using the standard curve method (boxplot, A) or using ImageJ (RT-PCR, B). CPEB2A/B ratio was determined to be significantly lower in tumor samples versus normal control tissue using the Mann-Whitney Rank Sum test (p=0.001). Outliers are indicated (•).

If our hypotheses are true, it is possible CPEB2 mRNA splicing is a central event in regulating the hypoxic response and acquisition of AnR in TNBC, opening doors to understanding a pivotal molecular component of cancer metastasis.

References:

1. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011; 121(7):2750-67. PMID: 21633166
2. http://seer.cancer.gov/statfacts/html/breast.html. Last accessed 1-6-14.
3. Howe EN, Cochrane DR, Cittelly DM, Richer JK. miR-200c targets a NF- κ B up-regulated TrkB/NTF3 autocrine signaling loop to enhance anoikis sensitivity in triple negative breast cancer. PLoS One. 2012;7(11):e49087. PMID: 23185507
4. Kurihara Y, Tokuriki M, Myojin R, Hori T, Kuroiwa A, Matsuda Y, Sakurai T, Kimura M, Hecht NB, Uesugi S. CPEB2, a novel putative translational regulator in mouse haploid germ cells. Biol Reprod. 2003 ;69(1):261-8.
5. Hägele S, Kühn U, Böning M, Katschinski DM. Cytoplasmic polyadenylation-element-binding protein (CPEB)1 and 2 bind to the HIF-1alpha mRNA 3'-UTR and modulate HIF-1alpha protein expression. Biochem J. 2009;417(1):235-46
6. Nairismägi ML, Vislovukh A, Meng Q, Kratassiouk G, Beldiman C, Petretich M, Groisman R, Füchtbauer EM, Harel-Bellan A, Groisman I. Translational control of TWIST1 expression in MCF-10A cell lines recapitulating breast cancer progression. Oncogene. 2012 Nov 22;31(47):4960-6. PMID: 22266852

Anoikis-Resistant (AnR) TNBC cells (able to survive off base tumor membrane) confirmed and matched with parental tissue (Par)

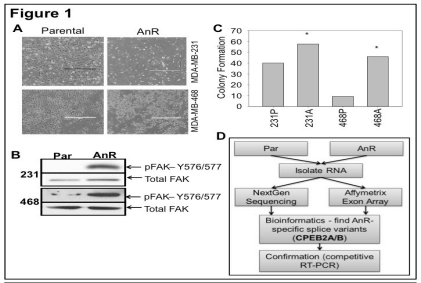


Figure 1: Development of AnR cell lines: MDA-MB-231 and MDA-MB-468 breast cancer cells were plated on poly-HEMA coated dishes and anoikis-resistant cells were selected for a period of about 3-5 weeks. **A.** Micrographs of parental and AnR cells **B.** Both parental (Parental, Par) and anoikis-resistant (AnR) cells were probed for phosphorylated FAK (Y576/577), a known marker for anoikis-resistance, and **C.** subjected to clonogenic analysis. Graph represents raw number of colonies (* = p<0.05 compared to control). **D.** After developing AnR cell lines, parental (Par) and anoikis resistant (AnR) cell lines were subjected to both RNAseq (NextGen Sequencing) and exon array analysis followed by bioinformatic analysis to identify common splice variants, which were dysregulated by the acquisition of AnR (experiments outlined in **D**).

Downregulation of CPEB2 isoforms

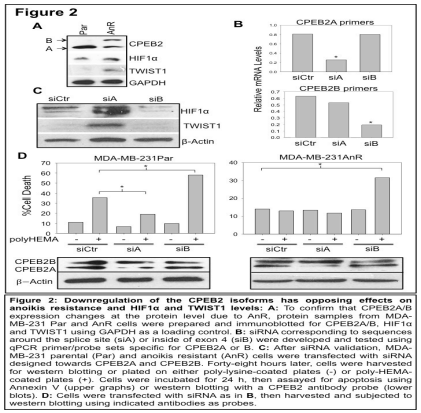


Figure 2: Downregulation of the CPEB2 isoforms has opposing effects on anoikis resistance and HIF1α and TWIST1 levels: A: To confirm that CPEB2A/B expression changes at the protein level due to AnR, protein samples from MDA-MB-231 Par and AnR cells were prepared and immunoblotted for CPEB2A/B, HIF1α and TWIST1 using GAPDH as a loading control. **B:** siRNA corresponding to sequences around the splice site (siA) or inside of exon 4 (siB) were developed and tested using qPCR primer/probe sets specific for CPEB2A or B. **C:** After siRNA validation, MDA-MB-231 parental (Par) and anoikis resistant (AnR) cells were transfected with siRNA designed towards CPEB2A and CPEB2B. Forty-eight hours later, cells were harvested for western blotting or plated on either poly-lysine-coated plates (-) or poly-HEMA-coated plates (+). Cells were incubated for 24 h, then assayed for apoptosis using Annexin V (upper graphs) or western blotting with a CPEB2 antibody probe (lower blots). **D:** Cells were transfected with siRNA as in **B**, then harvested and subjected to western blotting using indicated antibodies as probes.

Sponsored by: NIH/NCCAT #8KL2TR000057-03

Virginia Commonwealth University