Kentucky Journal of Undergraduate Scholarship

Volume 5 | Issue 1 Article 2

6-2021

Effect of HER2 Expression on NUPL2 Protein in Cervical Cancer Cells

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Cover Page Footnote

We would like to acknowledge our funding sources with support from Brescia University and a US Department of Education Title III Strengthening Institutions Program Grant.

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Abstract: *Recurring cervical cancer patients frequently present overexpressed Human Epidermal Growth Factor Receptor 2 (HER2) protein, which promotes the growth of the cancer. Some HER2-expressing cells and cervical cancer patients have enhanced gene expression of Nucleoporin-like 2 (NUPL2). As part of the nuclear pore complex, the NUPL2 protein serves to selectively export substances from the nucleus to the cytoplasm. In this experiment, HER2 was expressed in cervical cancer cells to examine its effect on NUPL2 protein expression and localization. Interestingly, there was no difference in NUPL2 protein levels between HER2-expressing and non-expressing cells. Importantly, consistent instances of NUPL2 protein localization specific to the nucleus occurred in a large sampling of HER2-expressing cells. This is significant because it suggests a mechanism as to how HER2 promotes excessive cell growth, via changes in NUPL2 localization, potentially impacting its function and ability to selectively export substances that modulate cell growth.*

Keywords: *HeLa Cells, nucleoporins, nucleus, immunoblot, immunofluorescence*

Cervical cancer is the fourth leading cause of cancer deaths in women worldwide (Bray et al., 2018). Frequently, patients with recurring cervical cancer have highly expressed Human Epidermal Growth Factor Receptor 2 (HER2) protein (Chavez-Blanco et al., 2004), a documented powerful cancer driver (Moasser, 2007). Many types of cancers are promoted by deregulating cellular nucleoporins, which are proteins of the nuclear pore complex involved in the transportation of gene transcripts, regulation of transcription, and chromatin access (Köhler & Hurt, 2010; Kau et al., 2004). Importantly, one of these nucleoporins, *Nucleoporin-like 2* (*NUPL2*), shows enhanced gene expression in HER2-positive breast cancer cell lines and tissues (Kalari et al., 2013; Wilson et al., 2002). No published data sets exist comparing HER2-positive cervical cancers and *NUPL2* gene expression; however, cervical cancer patient samples do present significantly higher *NUPL2* gene expression compared to control samples via microarray analysis (Long et al., 2017). Additionally, the RNA transport pathway (of which NUPL2 protein is a member) was found to be a key mechanism involved in the initiation, progression, and metastasis of cervical cancer (Long et al., 2017).

Interestingly, the *NUPL2* gene is amplified in 1% of cervical cancer patients and shows higher gene expression levels compared to controls according to cBioPortal for Cancer Genomics (Cerami et al., 2012; Gao et al., 2013). However, cervical squamous cell carcinoma patients show no significant difference in *NUPL2* gene expression between cancer grades, stages, nor survival according to UALCAN web-portal (Chandrasekhar et al., 2017). Together, these large data set studies have suggested a possible link between HER2 expression and NUPL2 protein in cervical cancer, but this connection has not been reported. Based upon the data independently demonstrating cervical cancer and HER2-expression changes to gene expression of *NUPL2*, and the importance of NUPL2 localization for its function, here we examined the protein levels of NUPL2 with immunoblot and its localization via immunofluorescence in response to HER2 overexpression in HeLa cells. In the current study, we sought to examine whether the expression of HER2 could modify the protein expression, or the location of NUPL2 protein, or both, and as a result, could impact the transport, regulation, and access of genes involved in cancer growth.

Method

Cell Culture

HeLa cells were grown in DMEM with Glutamax (Gibco 10565018) supplemented with Fetal Bovine Serum (10%) (FBS) (Atlanta Biologicals) and Antibiotic-Antimycotic (Gibco 15240062). For immunoblot experiments, cells were plated at 70,000 cells/well on a 6 cm plate. For immunofluorescence experiments, cells were plated at 10,000 cells/well on an 8-well chamber slide. For both experiments, cells were grown for 24 hours. Plasmid DNA, either control pcDNA3.1-EGFP or pERBB2-EGFP (Addgene plasmid # 39321), was transfected with Lipofectamine 3000 (ThermoFisher Scientific L3000001) via manufacture instructions. Cells were allowed to grow for 24 hours then used for assays.

Immunoblot

HeLa cells on 6 cm plates were washed twice with phosphate buffered saline (PBS) on ice and scraped into a microcentrifuge tube. Cells were microcentrifuged at 4 ˚C at 600 X g for 2 minutes and then the supernatant was discarded. The cell pellets were resuspended with a RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.5% Sodium-deoxycholate, 0.1% SDS, 150 mM NaCl, 2 mM EDTA pH 8.0, 50 mM NaF) supplemented with a protease inhibitor cocktail (Sigma S8820-2TAB) then passed 50 times with a P200 micropipette. Samples were placed on dry ice for 2 minutes, then removed from dry ice and warmed quickly. Samples were passed 50 times again with a P200 micropipette and placed back on dry ice for 2 minutes. Samples were warmed quickly and then placed on ice for 10 minutes. Samples were microcentrifuged at 4 ˚C for 10 minutes at 20,000 X g. The supernatant was removed and used with common SDS-PAGE and liquid transfer of proteins on nitrocellulose protocols. Anti-GFP antibody (Sigma G6539) was used at a 1:1000 dilution in PBS-Tween. Anti-NUPL2 antibody (Abcam EPR16545) was used at a 1:1000 dilution in PBS-Tween. Anti-GAPDH antibody (Cell Signaling 5174T) was used at a 1:1000 dilution in PBS-Tween. All antibodies were incubated with nitrocellulose blots overnight on a rocking platform at 4 ˚C. Anti-Rabbit and anti-Mouse IgG HRP-linked antibodies (Cell Signaling 7074 and 7076) were used at 1:1000 dilutions and incubated at room temperature for 30 minutes on a rocking platform. HRP Conjugate Substrate Kit (BioRad 1706431) was used to detect HRP secondary antibodies via manufacturer directions. Images were acquired using the gel imaging system (BioRad Gel Doc 2000 Chemi Doc 1708126).

Immunofluorescence

HeLa cells on chamber slides were fixed with a 4% paraformaldehyde solution in PBS for 10 minutes at room temperature. Cells were then permeabilized with a 0.5% Tween-20 in PBS solution for 10 minutes at room temperature. Finally, cells were blocked in a 3% Goat Serum (Sigma G9023) in PBS overnight at 4 ˚C. Prior to each step above, cells were washed 3 times for 3 minutes each with PBS. Fixed cells were then incubated with the primary antibody NUPL2 (Abcam EPR16545) diluted 1:100 in 3% Goat Serum for 1 hour at 4 ˚C. Secondary antibody Goat anti-Rabbit Alexa Fluor 594 (ThermoFisher R37177) was diluted 1:500 in 3% Goat Serum and incubated for 30 minutes at 4 ˚C in dark. DAPI Stain (Chemical FL 341 Blue) (Pierce 62248) was diluted to a final concentration 1 µg/mL and applied to each well for 8 minutes at room temperature in dark. Between each step above, wells were washed 3 times with PBS. Chamber slides were dismantled and washed twice gently with ultrapure water. Slides were dried and mounted with coverslips with ProLong Glass Antifade Mountant (Invitrogen P36982) per manufacturer directions. Slides were imaged using the EVOS FL imaging system (Life Technologies). EVOS LED GFP light cube (AMEP4651) was used to analyze GFP-expressing HeLa cells. EVOS LED TexasRed light cube (AMEP4655) was used to analyze NUPL2/Alexa Fluor 594. EVOS LED DAPI light cube (AMEP4650) was used to analyze DAPI Stain. Analysis of immunofluorescence data was completed using ImageJ (https://imagej.nih.gov/ij/download/).

Results

Since several studies indicate changes to *NUPL2* gene expression in HER2-positive breast samples (Kalari et al., 2013; Wilson et al., 2002), and in cervical cancer patients (Long et al., 2017), the protein levels of NUPL2 were examined via immunoblot. NUPL2 protein levels remained unchanged between HER2-expressing and non-expressing HeLa cells (Figure 1). This indicates an inability for high levels of HER2 to compromise the normal protein expression or stability of NUPL2 in these cells.

Previous work showed that NUPL2 protein localized primarily around the nucleoplasm, and some cytoplasmic staining in HeLa cells according to The Human Protein Atlas (Thul et al., 2017). NUPL2 presents its functionality in the nucleus as a part of the nuclear pore complex (Köhler & Hurt, 2010); thus immunofluorescence was used to examine the localization of NUPL2 in HER2-expressing cells (Figure 2). Importantly, NUPL2 localized within the nucleus in 65% of cells expressing HER2 (N = 100), compared to just 20% access in the non-expressing cells (N = 180) (Table 1). Interestingly, only in HER2-expressing cells did NUPL2 exclusively localize to the nucleus. There was a decreased corresponding 56% change in cytosolic localization of NUPL2 in the HER2-expressing cells compared to non-expressing cells. Together these data indicate a change in localization of NUPL2 more towards the nucleus in HER2-expressing cells.

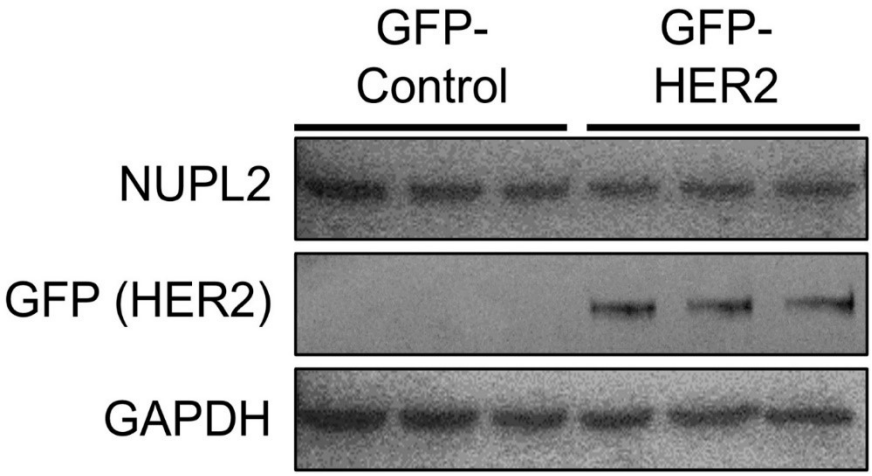


Figure 1. Immunoblot detection of NUPL2 protein in HER2-expressing HeLa cells. HeLa cells transfected with GFP-tagged vector control or with GFP-tagged HER2 were then harvested for protein and used for immunoblot using antibodies specific for NUPL2, GFP, and GAPDH. Three independent samples are provided per condition.

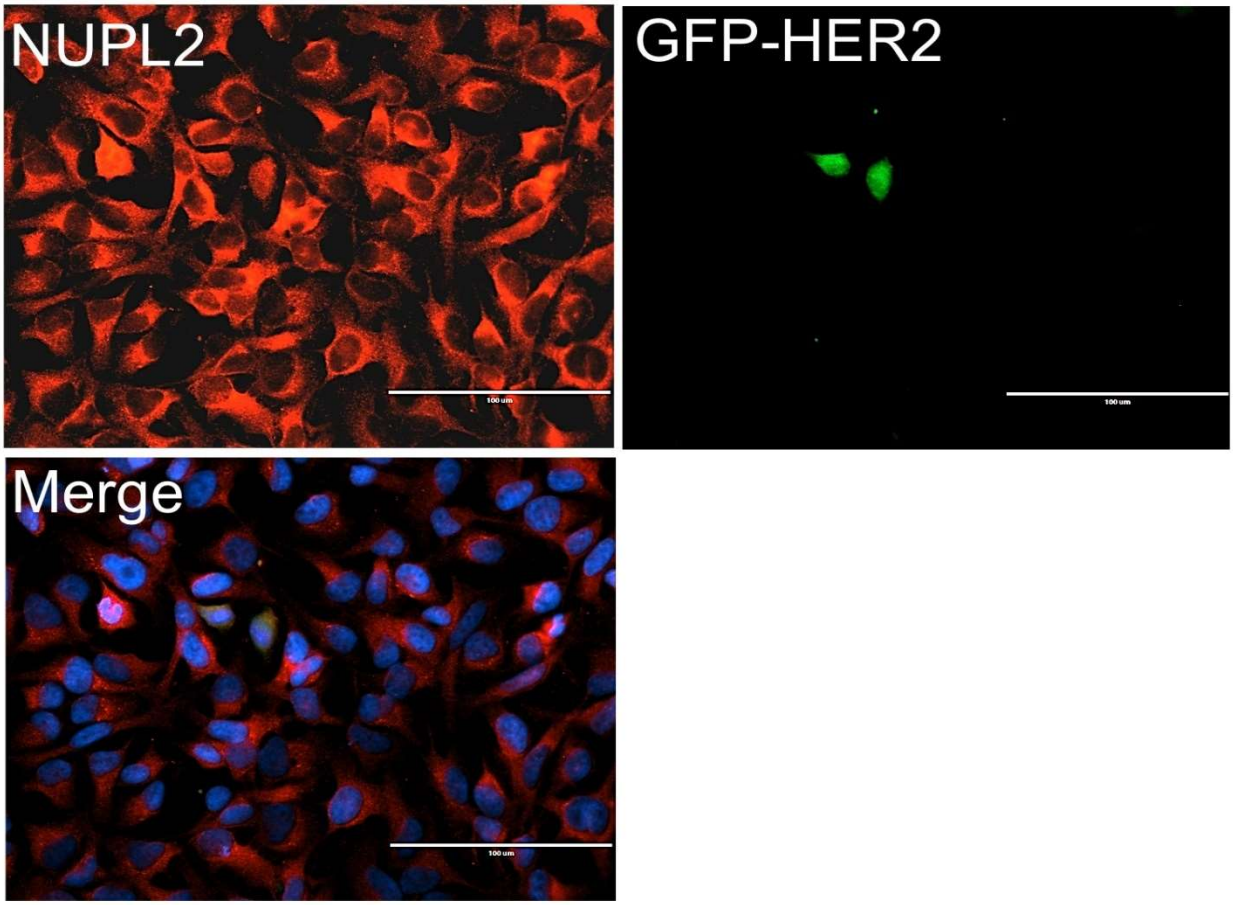


Figure 2. Immunofluorescence detection of NUPL2 protein in HER2-expressing HeLa cells. HeLa cells transfected without or with GFP-tagged HER2 (green) using an antibody for NUPL2 (red). Cells were co-stained for nuclei (blue) in overlay with the other two channels (merge).

Published by Encompass, 2021

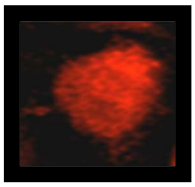
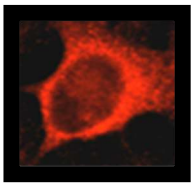
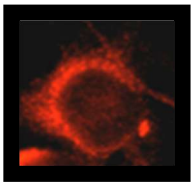
	Nucleus Only	*Both*	*Cytosol Only*
Control (N = 180)	0 %	20 %	80 %
HER2 (N = 100)	14 %	51 %	35 %
			

Table 1. NUPL2 localization in HER2-expressing HeLa cells. The quantification of localization of NUPL2 (red) in HeLa cells transfected without (Control) (N = 180 cells) or with HER2 (N = 100 cells). Cells were analyzed by three separate investigators in the blind. Nucleus Only = residing visibly only in nucleus; Cytosol only = residing visibly only outside the nucleus; Both = residing visibly in and out of the nucleus. Representative images of NUPL2 localization for each group are provided.

Discussion

Prior to this investigation, there were no published studies examining the impact of HER2 expression on NUPL2 protein in any cell line. In fact, in cervical cancer cells, NUPL2 protein levels and localization have only been reported previously in HeLa cells found on (Abcam EPR16545) and The Human Protein Atlas (Thul et al., 2017), and not in cervical cancer cell lines expressing high HER2 (SiHa, C33A, CaSki) (Narisawa-Saito et al., 2006). Future studies on HER2 in cervical cancer might examine these cell lines with higher HER2 expression for NUPL2 protein to see if there are relative change in basal levels. This study is the first to characterize the role of HER2 expression on NUPL2 protein with immunoblot and immunofluorescence data. The data clearly demonstrates that expression of HER2 does not influence the protein levels of NUPL2 in HeLa cells. This is critical information for the field, as previous studies demonstrated a change in the *NUPL2* gene expression levels in HER2-positive breast tissues via cDNA microarray and RNA-Seq data sets (Wilson et al., 2002; Kalari et al., 2013) and in cervical cancer patient samples (Long et al., 2017). It is likely that other unknown tissue- and organ-specific factors such as hormones or post-translational processing may be important to the regulation of NUPL2 protein production, of which HER2 may be a corresponding factor.

The immunofluorescence data paints an intriguing role of HER2 expression in the localization of NUPL2 protein. Studies have shown that when NUPL2 protein and other nucleoporins are deregulated, these proteins function to promote cell growth and cancer proliferation (Nofrini et al., 2016). The localization change of NUPL2 to the nucleus suggests that HER2 expression could impact the functionality of NUPL2 in HeLa cells. Interactome enrichment studies have shown that other nucleoporins could be deregulated in HER2-positive tumors (Kalari et al., 2013; Long et al., 2017). In conclusion, these findings provide initial information about the relationship between NUPL2 protein and HER2 expression in cells. This data provides the foundational knowledge to explore NUPL2 and other nucleoporins and isolate their relationships in HER2-positive recurring cervical cancer patients. Perhaps NUPL2 could indicate a viable mechanistic target for understanding and unlocking new nucleoporin-related gene therapy options.

References

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A. & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6): 394-424. doi:10.3322/caac.21492

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., . . . Schultz, N. (2012). The CBIO cancer genomics portal: An open platform for

exploring multidimensional cancer genomics data: Figure 1. *Cancer Discovery, 2*(5), 401-404. doi:10.1158/2159-8290.cd-12-0095

Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B. V., & Varambally, S. (2017). UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia, 19*(8), 649-658. doi:10.1016/j.neo.2017.05.002

Chavez-Blanco, A., Perez-Sanchez, V., Gonzalez-Fierro, A., Vela-Chavez, T. Candelaria, M., Cetina, L., … Dueñas-Gonzalez, A. (2004). HER2 expression in cervical cancer as a potential therapeutic target. *BMC Cancer*, *4*(1). doi:10.1186/1471-2407-4-59

Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., . . . Schultz, N. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cbioportal. *Science Signaling, 6*(269). doi:10.1126/scisignal.2004088

Kalari, K. R., Necela, B. M., Tang, X., Thompson, K. J., Lau, M., Eckel-Passow, J. E., … Perez, E. A. (2013). An integrated model of the transcriptome of her2-positive breast cancer. *PLoS ONE*, *8*(11). doi.org/10.1371/journal.pone.0079298

Kau, T. R., Way, J. C. & Silver, P. A. (2004). Nuclear transport and cancer: From mechanism to intervention. *Nature Reviews Cancer 4*(2), 106–117. doi.org/10.1038/nrc1274

Köhler, A., & Hurt, E. (2010). Gene regulation by nucleoporins and links to cancer. *Molecular Cell, 38*(1), 6-15. doi:10.1016/j.molcel.2010.01.040

Long, N. P., Jung, K. H., Yoon, S. J., Anh, N. H., Nghi, T. D., Kang, Y. P., H. H., Min, J. E., Hong, S. S., & Kwon, S. W. (2017). Systematic assessment of cervical cancer initiation and progression uncovers genetic panels for deep learning-based early diagnosis and proposes novel diagnostic and prognostic biomarkers. *Oncotarget*, *8*(65), 109436-109456. doi.org/10.18632/oncotarget.22689

Moasser M. M. (2007). The oncogene HER2: Its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene*, *26*(45), 6469–6487. doi.org/10.1038/sj.onc.1210477

Narisawa-Saito, M., Handa, K., Yugawa, T., Ohno, S., Fujita, M., & Kiyono, T. (2006). HPV16 E6-mediated stabilization of ErbB2 in neoplastic transformation of human cervical keratinocytes. *Oncogene, 26*(21), 2988-2996. doi:10.1038/sj.onc.1210118

Nofrini, V., Giacomo, D. D., & Mecucci, C. (2016). Nucleoporin genes in human diseases. *European Journal of Human Genetics*, *24*(10), 1388–1395. doi.org/10.1038/ejhg.2016.25

Thul, P. J., Åkesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Blal, H. A., . . . Lundberg, E. (2017). A subcellular map of the human proteome. *Science, 356*(6340). doi:10.1126/science.aal3321

Wilson, K. S., Roberts, H., Leek, R., Harris, A. L., & Geradts, J. (2002). Differential gene expression patterns in her2/neu-positive and -negative breast cancer cell lines and tissues. *The American Journal of Pathology, 161*(4), 1171-1185. doi:10.1016/s0002-9440(10)64394-5