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14. ABSTRACT

In previous years of this project (2006-2008); we have identified a novel mechanism of internalization and trafficking for the epidermal growth factor receptor in breast cancer cells that overexpress the MUC1 oncoprotein (El Bejjani et al 2006). Additionally, I showed that in the presence of MUC1, EGFR is trafficked to the endocytic recycling compartment and not to the late endosome. This provides an explanation for the decrease in EGFR degradation and increase in signaling observed in MUC1-expressing breast cancer cells. Additionally, I identified a novel non-canonical EGFR trafficking mechanism in MUC1 overexpressing breast cancer cells. I show that following EGF-induced internalization, EGFR is trafficked to the early endosome regardless of MUC1 expression. Interestingly, in later time points, we observed that EGFR is colocalized with late endosomal markers only when MUC1 expression is knocked down with siRNA. In cells that express high levels of MUC1 protein, I observed a colocalization of MUC1 and EGFR at the endocytic recycling compartment (ERC) and not at the late endosome. Furthermore, I showed that localization of EGFR to the ERC affects EGFR signaling through the pSTAT3 pathway but not through the pAKT or dpERK pathways. These results consists a major part of a publication still in preparation in Dr Joyce Schroeder's lab which will be submitted after my graduation.

15. SUBJECT TERMS

MUC1, EGFR, Endocytosis, Degradation, Ubiquitination, Recycling

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INTRODUCTION:

EGFR is a member of the ErbB family of receptor tyrosine kinases. Ligand binding to this receptor induces phosphorylation of the receptor and activation of its kinase domain (1). Activation of the receptor induces signaling that leads to cell growth, proliferation, migration and inhibition of apoptosis. Activated EGFR is internalized and in normal cases shuttled from the early endosome to the late endosome and eventually to the lysosome where it is degraded (2). MUC1 is a highly glycosylated membrane protein. It is overexpressed in 90% of breast cancer and has been shown to cause metastatic breast cancer when ectopically overexpressed in mouse mammary glands (3). We have previously reported that MUC1 expression inhibits the degradation of EGFR while promoting its internalization. This is accompanied by increased EGFR recycling and signaling which could have tumorigenic properties (4). In our last months for this project we identified a novel trafficking pathway for EGFR in cells that overexpress MUC1. We will show that EGFR is trafficked in a non-canonical; manner towards the endocytic recycling compartment (ERC) instead of the accepted trafficking towards the Rab-7 containing late endosome preceding degradation in the lysosome (see Chapter III of dissertation in appendices).

BODY:

We proposed to determine the identity of the perinuclear colocalization compartment of MUC1 and EGFR in the presence of EGF. We had previously observed a strong colocalization of EGFR and MUC1 in a perinuclear compartment in breast cancer cells by deconvolution and conventional fluorescence microscopy (see preliminary data and (4)). We set out to identify this compartment within breast cancer cells. We utilized plasmids encoding fluorescently tagged constructs of Rab11-Dsred and RME-1-GFP that label the endocytic recycling endosome. Additionally we made use of Rab5-GFP constructs to label early endosomes and Rab-7 CFP to label late endosomes. Using these techniques, we assessed the localization of MUC1 and EGFR when cells are treated with MUC1 siRNA or control siRNA and when cells are grown in the absence of EGF or after EGF stimulation in BT20 and in MDA-MB-468 breast cancer cell lines. Additionally, we obtained dominant negative constructs of RME-1 generously provided by Dr Barth Grant at Rutgers University. Transfection of these constructs in Hela Cells and in C elegans disrupts the function of the ERC (8).

The experiments provided in detail in the chapter III of my dissertation identified a novel, non-canonical EGFR trafficking pathway in MUC1 overexpressing breast cancer cells. In the presence of MUC1, EGF stimulation induces the enhanced internalization of EGFR. Internalized EGFR is then trafficked into the Rab-5 positive early endosome. Trafficking of EGFR to the early endosome does not depend on MUC 1 expression and has been previously reported. In canonical EGFR trafficking, after being transported to the early endosome, internalized receptors are then trafficked to the late endosome from which they will be shuttled to the lysosome leading to degradation and termination of a wave of EGFR signaling. We observed that, in the presence of MUC1, EGFR is not found at the Rab-7 positive late endosome but is instead trafficked to the Rab-11 and RME-1 positive ERC. Localization of EGFR at the ERC supports our previous findings showing that in the presence of MUC1, EGFR is recycled back to the plasma membrane rather than degraded after EGF stimulation (4).

Additionally, we investigated the role of this novel, MUC1-dependent EGFR localization at the ERC in EGFR signaling. We transfected cells with RME-1 dominant negative constructs in the presence or absence of MUC1. We showed that disruption of ERC function does not affect AKT or MAPK signaling following EGF stimulation. However, we determined that STAT3 is activated in response to EGF in MUC1-overexpressing cells. Additionally we observed a time dependent downregulation of pSTAT3 after EGF stimulation. We show that MUC1 expression inhibits pSTAT3 downregulation and that dominant-negative RME-1 (RME-1G65R) shows an additive effect with MUC1 siRNA and further accelerates pSTAT3 downregulation. Detailed description of the results, methods and data for the above mentioned study is available as the third chapter of my disseration referred to in the appendices section. This study is being completed for submission as a manuscript in the near future.

KEY RESEARCH ACCOMPLISHMENTS:

- MUC1 induces EGFR trafficking to the perinuclear recycling endosome
- MUC1 knockdown does not affect early endosome localization
- MUC1 knockdown restores EGFR trafficking to Rab7-positive late endosomes
- MUC1-induces EGFR trafficking to RME-1 –positive ERC
- Dominant negative RME-1 affects pSTAT3 but not pAKT and dpERK signaling downstream of EGFR.

REPORTABLE OUTCOMES:

Rachid El Bejjani and Joyce A Schroeder. Effect OF MUC1 expression on EGFR endocytosis and degradation in Human breast cancer cell lines (Poster to be presented at the Era of Hope meeting June 2008 Baltimore, MD)

Rachid El Bejjani and Joyce Schroeder. MUC1 redirects the Epidermal growth factor receptor towards the ERC and affects its signaling *(Manuscript in preparation)*

Rachid El Bejjani. MUC1 alter EGFR trafficking and signaling in breast cancer cells. (completed dissertation submitted to the faculty of the department of molecular and cellular biology at the University of Arizona)

CONCLUSION:

The research I conducted for my PhD dissertation and supported in part by this grant has broadened our understanding of the field of MUC1 biology and EGFR trafficking. In this work, we have demonstrated an important role for MUC1 in regulating EGFR trafficking. We showed that MUC1 expression can enhance the internalization of EGFR while inhibiting its ubiquitination. This results in a decrease in the degradation of the receptor and in an increased recycling in response to EGF. We then investigated the mechanism behind MUC1-dependent EGFR trafficking. We showed that, when MUC1 is present, EGFR colocalizes with MUC1 at the Rab11 and RME-1 positive perinuclear endocytic recycling compartment (ERC). Interestingly, MUC1 expression did not significantly alter the localization of EGFR at the Rab5-positive early endosome; However, MUC1 knockdown restored EGFR trafficking towards Rab7-positive late endosomes and away from the perinuclear ERC. These results describe a novel MUC1- dependent EGFR trafficking pathway in breast cancer cells. our results identify MUC1 as a modulator of EGFR trafficking and describe a novel MUC1-dependent EGFR trafficking pathway. This altered EGFR trafficking results in enhanced EGFR activation which could have significant implications for breast cancer biology and therapy.

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APPENDIX

Due to a lack of space, I will not attach the document for my complete dissertation to this report. The complete document can be found in pdf format free of charge at the following web address.

http://etd.library.arizona.edu/etd/metadata.jsp?id=urn:etd:azu_etd_10004_sip1_m