Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Metabolic glycoengineering sensitizes drug-resistant pancreatic cancer cells to tyrosine kinase inhibitors erlotinib and gefitinib

Mohit P. Mathew [†], Elaine Tan [†], Christopher T. Saeui, Patawut Bovonratwet, Lingshu Liu, Rahul Bhattacharya, Kevin J. Yarema [*]

*Department of Biomedical Engineering and the Translational Tissue Engineering Center, The Johns Hopkins University, 5029 Robert H. & Clarice Smith Building, 400 North Broadway, Baltimore, MD 21231, USA*

ARTICLE INFO

*Article history:*
Received 5 January 2015
Revised 23 January 2015
Accepted 26 January 2015
Available online xxxx

*Keywords:*
Metabolic oligosaccharide engineering
Epidermal growth factor receptor
Sialylation
Receptor tyrosine kinase inhibitor
Drug synergy
Pancreatic cancer

ABSTRACT

Metastatic human pancreatic cancer cells (the SW1990 line) that are resistant to the EGFR-targeting tyrosine kinase inhibitor drugs (TKI) erlotinib and gefitinib were treated with 1,3,4-*O*-$Bu_3$ManNAc, a 'metabolic glycoengineering' drug candidate that increased sialylation by ~2-fold. Consistent with genetic methods previously used to increase EGFR sialylation, this small molecule reduced EGF binding, EGFR transphosphorylation, and downstream STAT activation. Significantly, co-treatment with both the sugar pharmacophore and the existing TKI drugs resulted in strong synergy, in essence re-sensitizing the SW1990 cells to these drugs. Finally, 1,3,4-*O*-$Bu_3$ManNAz, which is the azido-modified counterpart to 1,3,4-*O*-$Bu_3$ManNAc, provided a similar benefit thereby establishing a broad-based foundation to extend a 'metabolic glycoengineering' approach to clinical applications.



Tumor-associated carbohydrate antigens (TACAs) have been associated with cancer for decades[1,2] and abnormal glycosylation is now accepted to be a universal feature of cancer.[3] Despite the many roles glycosylation has been discovered to play in cancer progression and metastasis,[4–6] progress in exploiting this knowledge in a clinical setting has been agonizingly slow.[7,8] The epidermal growth factor receptor (EGFR) exemplifies the 'sweet and sour'[8] or 'bittersweet'[7] nature of glycosylation in cancer. On one hand, several papers over the past ~15 years have reported that modulation of EGFR's glycosylation status—in particular changes to fucose and sialic acid[9]—control this receptor's ability to drive cancer progression. For example, a recent report showed that increased sialylation or fucosylation of EGFR suppressed dimerization, inhibited subsequent phosphorylation, and dampened activation of downstream signaling in lung cancer cells.[9] On the other hand, although these responses would be expected to slow cancer cell growth, the methods used to demonstrate these features in cell culture experiments, such as enzymatic removal of sugars or genetic over-expression of glycosyltransferases, are not easily translated to animal testing or to a human clinical setting thereby illustrating the general difficulties in developing carbohydrate-based cancer therapies.

In this report we overcome a hurdle towards clinical exploitation of the glycosylation status of EGFR by using small molecule 'metabolic glycoengineering' sugar analogs to increase the sialylation of this receptor and reduce its activity in ways that were previously only accomplished genetically.[9,10] Metabolic glycoengineering[11] (also referred to as metabolic oligosaccharide engineering or MOE[12,13]) is a versatile technique used to change patterns of glycosylation by altering the availability or chemical composition of biosynthetic precursors of glycans.[14,15] For example, short chain fatty acid (SCFA)-modified ManNAc analogs can efficiently enhance metabolic flux through the sialic acid biosynthetic pathway[16,17] and increase cell surface sialylation.[18] The ester-linked SCFA moieties, which are usually acetate[19–21] or *n*-butyrate,[17,22] increase cellular uptake by three orders of magnitude or more and upon entering a cell, intracellular esterases rapidly remove the SCFA groups,[23] regenerating the core sugar that enters the targeted biosynthetic pathway. This strategy positions SCFA-modified sugars as viable drug candidates, as evidenced by the recent use of peracetylated ManNAc ($Ac_4$ManNAc, shown in Fig. 1) to reverse the symptoms of hereditary inclusion body myopathy in an animal model of this disease.[24]

One drawback of SCFA-conjugated monosaccharide drug candidates is cytotoxicity and growth inhibition that, while not severe, can hinder metabolic incorporation into cell surface glycans.[16,17,25] On the other hand, we have previously shown that cellular

* Corresponding author. Tel.: +1 410 614 6835; fax: +1 410 614 6840.
*E-mail address:* kyarema1@jhu.edu (K.J. Yarema).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2015.01.060
0960-894X/

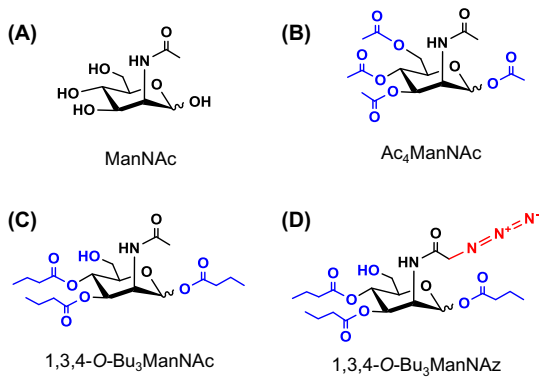


**Figure 1.** ManNAc and analogs used for metabolic glycoengineering pancreatic cancer cells for increased sensitivity to EGFR-targeting TKI drugs. (A) Natural ManNAc. (B) Fully acetylated ManNAc, $Ac_4$ManNAc. (C) 'High-flux' tributanoylated ManNAc, 1,3,4-*O*-$Bu_3$ManNAc. (D) Azide-modified high flux ManNAc, 1,3,4-*O*-$Bu_3$ManNAz.

responses elicited by SCFA-modified ManNAc,[17,22,26] which include down-regulation of NF-κB and metastatic oncogenes,[27] have potential anti-cancer properties.[13,28] Based on considerable evidence that *O*-acetylated sialic acids can play roles in cancer biology,[29–33] we were intrigued whether *n*-butyrate could be 'carried through' the biosynthetic pathway and appear on the cell surface as *O*-butanoylated siaolosides. Careful mass spectrometry profiling of sialic acids (e.g., as reported in a recent publication[34]) failed to support this hypothesis however, Instead, our laboratory has uncovered structure activity relationships (SARs) that attribute analog-mediated cytotoxicity and high levels of growth inhibition to the presence of a SCFA group at the C6 position of a hexosamine.[28,35] This discovery allowed us to develop tributanoylated sugars such as 1,3,4-*O*-$Bu_3$ManNAc (Fig. 1) that achieve high flux into the sialic acid pathway at low analog concentrations compared to the widely used peracylated compounds (e.g., $Ac_4$ManNAc).[18] Importantly, use of the '1,3,4' analogs at concentrations less than 100 μM allow the side effects discussed above (e.g., cytotoxicity, severe growth inhibition, or NF-κB inhibition) to largely be avoided thus enabling a 'glycan only' investigation of the impact of ManNAc analogs.

Of particular relevance to our efforts to develop glycosylation-based therapies for chemotherapy-resistant pancreatic cancer, we found that EGFR in SW1990 cells treated with 1,3,4-*O*-$Bu_3$ManNAc experienced an increase in sialylation of 2-fold or higher by using mass spectrometry-based 'glycosite'[36] and glycan analysis methods similar to those already reported[34,37] (relevant experimental

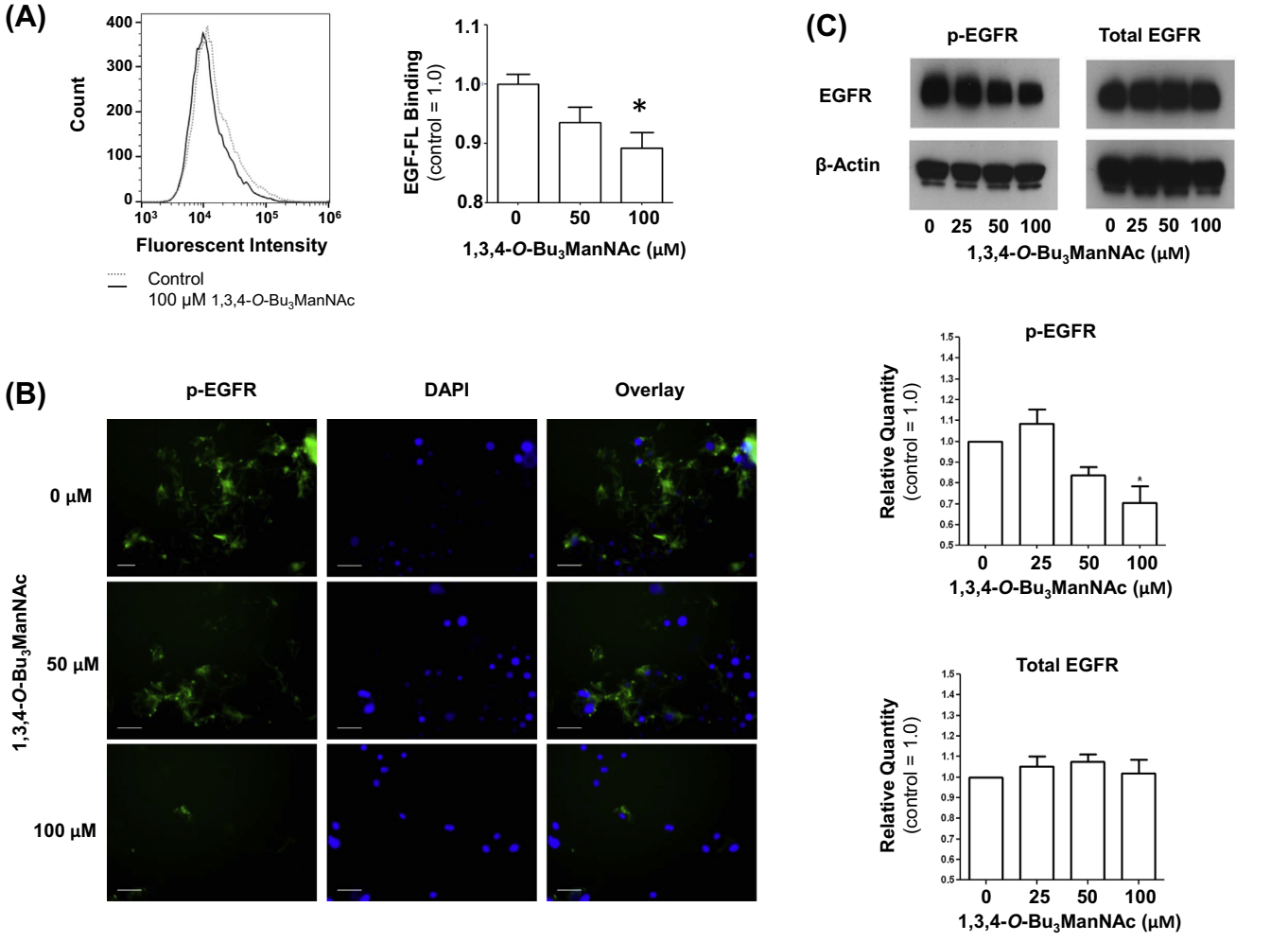


**Figure 2.** 1,3,4-*O*-$Bu_3$ManNAc decreases EGFR phosphorylation. (A) Saturation binding where cells were incubated with Alexa Fluor® 488 labeled EGF for one hour at room temperature and then measured using flow cytometry shows a decrease in available surface bound EGFR. At least 3 biological replicates were carried out for each experiment with data expressed as mean ± standard error mean (SEM). (B) Representative images of immunofluorescence assays (additional images are provided in Supplemental Fig. S1) where cells were incubated with EGF for 2.0 min, fixed and stained with anti-p-EGFR, FITC labeled anti-rabbit antibody, and stained with DAPI confirm that EGFR phosphorylation decreases with analog treatment. (C) Western blots of SW1990 pancreatic cancer cells treated with increasing levels of 1,3,4-*O*-$Bu_3$ManNAc showed a decrease in phosphorylated EGFR with no significant change in overall EGFR levels. * indicates a $p$ value of <0.05.