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AUTHOR'S VIEWS

Author's view: epithelial plasticity metabolically reprograms normal cells towards a neoplastic-prone state

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ABSTRACT

We have uncovered that epithelial plasticity programs metabolically reprogram epithelial lung cells by increasing expression of genes (e.g., *glutamine-fructose-6-phosphate transaminase 2 – GFPT2* and *UDP-N-acetylglucosamine pyrophosphorylase 1 – UAP1*) critical for the hexosamine biosynthetic pathway (HBP) and elevating global protein O-GlcNAcylation – a specific type of glycosylation. We found that increased O-GlcNAcylation could suppress oncogene-induced senescence tumor suppressor pathways that ultimately led to accelerated $Kras^{G12D}$-driven lung tumorigenesis.

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Altered metabolism is one of the hallmarks of the neoplastic phenotype. Cancer cells exhibit profound metabolic alterations to meet energy needs for growth and survival. The best-known cancer metabolic anomaly is an increase in aerobic glycolysis, which was first reported by Otto Warburg more than 80 years ago. Glycolysis plays fundamental roles in cancer cell growth and survival by directing carbon sources into multiple branching pathways for the de novo generation of nucleotides, lipids, proteins and molecular energy in the form of ATP. However, the role of these metabolic adaptations for early tumorigenesis are less well appreciated.

Epithelial-mesenchymal transition (EMT) is a crucial, evolutionarily conserved process that is essential for embryonic development. We and others have showed that EMT plays an important role not only during tumor progression towards metastasis and treatment resistance, but perhaps more importantly, during the earliest stages of tumorigenesis.[1,2] Cellular senescence and apoptosis elicited by oncoproteins such as $Kras^{G12D}$ represent early tumor suppressive barriers that must be overcome for pre-malignant cells to transform into cancer cells.[3] We and others have reported previously that EMT transcription factors (EMT-TFs) can suppress oncogene-induced senescence or apoptosis (OIS/OIA) *in vitro* and *in vivo* resulting in acceleration of tumorigenesis and tumor progression.[1,2] Mechanisms implicated for EMT-TF suppression of OIS/OIA involved inhibition of the p53 tumor suppressor (TP53, best known as p53).[4] More recent evidence links EMT-related transcriptomic alterations with metabolic reprograming in cancer cells, which include increased aerobic glycolysis and the accumulation of dihydropyrimidines.[5] In our recent study, we demonstrated that EMT driven by *Twist1* or *SNAI1*, two key regulators of EMT, metabolically reprogram epithelial lung cells and lung cancer cells by increasing expression for genes that encode the rate limiting step (*glutamine-fructose-6-phosphate transaminase 2 – GFPT2*) and final enzymatic step (*UDP-N-acetylglucosamine pyrophosphorylase 1 – UAP1*) of the hexosamine biosynthetic pathway (HBP). HBP stimulation increased global protein O-GlcNAcylation, a specific type of glycosylation, and ultimately accelerate $Kras^{G12D}$-driven lung tumorigenesis,[6](Figure 1).

The HBP is a side-branch from glycolysis, in which glucose is diverted at the fructose−6-phosphate step.[7] The HBP serves as a sensor hub of cellular metabolism and integrates the flux of metabolites through many metabolic pathways linked to nutrient intake. Glucose, glutamine and fatty acids are substrates that generate the final product of the HBP, UDP-N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is a basic building block of protein N- and O-glycosylations, two important post-translational modifications (PTMs) identified in many proteins in the cell cytoplasm, nucleus, mitochondria and cell membrane. Variations in the levels of glucose, glutamine and fatty acids in the cells affect the flux through the HBP pathway and the production of UDP-GlcNAc. Therefore UDP-GlcNAc can be viewed as a molecular thermostat for nutrient levels. A key downstream utilization of UDP-GlcNAc is O-GlcNAcylation which is a nutrient- and stress-responsive PTM that involves the attachment of the GlcNAc moiety to Ser, Thr and Asn residues of cytoplasmic, nuclear and mitochondrial proteins. A single pair of enzymes – O-GlcNAc transferase (*OGT*) and O-GlcNAcase (*OGA*) – controls the addition and removal of O-GlcNAc. O-GlcNAcylation competes with phosphorylation on Ser/Thr and thus regulates diverse cellular processes, which include transcription, protein stability and cell signaling dynamics. Global changes in protein O-GlcNAcylation is emerging as a general characteristic of cancer cells. In our study, we demonstrated that the EMT-HBP-O-GlcNAcylation

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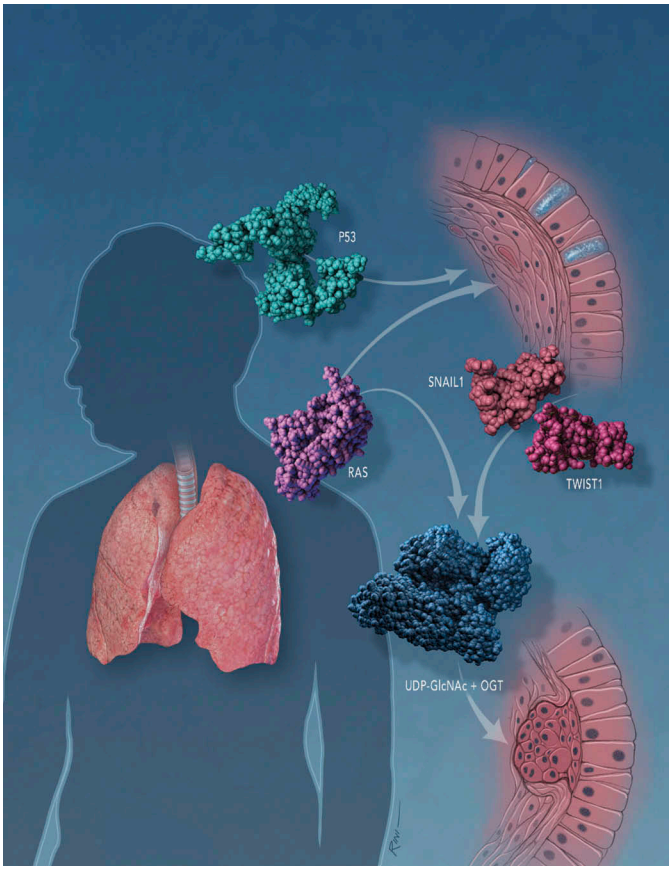

Figure 1. EMT-TFs metabolically reprogram normal lung epithelial cells towards a neoplastic-prone state. Upper-right panel – Aberrantly activated mutant RAS protein (purple colored protein) leads to premature cellular senescence of lung epithelial cells as shown by blue cells that is enforced by p53 (TP53, best known as p53, aqua colored protein)-dependent oncogene-induced senescence. Lower-right panel – epithelial-mesenchymal transition-transcription factors (EMT-TFs) TWIST1 and SNAIL1 (violet and salmon colored proteins, respectively) reprogram epithelial metabolism towards a tumorigenic permissive state by upregulation of the final product of the hexosamine biosynthesis pathway, UDP-N-acetylglucosamine (UDP-GlcNAc), and the O-GlcNAc transferase (OGT) – the enzyme responsible for adding GlcNAc molecules onto proteins (blue colored protein). This metabolic shift leads to increased global O-GlcNAcylation that in the background of activated mutant RAS protein (purple colored protein) results in increased $Kras^{G12D}$-induced lung tumorigenesis (neoplastic collection of cells invading through the basement membrane).

axis drives the O-GlcNAcylation of key proteins such as c-Myc, which has been shown previously to suppress $Kras^{G12D}$ -induced OIS and contribute to increased tumorigenesis.[8] In addition, O-GlcNAcylation has been previously reported to stabilize c-Myc protein,[9] therefore, O-GlcNAcylated c-Myc potentially plays a causal role in EMT-driven OIS suppression and resultant increased tumorigenesis.

Interestingly, mutant $Kras^{G12D}$ itself had also been shown previously to drive increased flux through the HBP in an inducible mouse model of pancreatic ductal adenocarcinoma.[10] However, the requirement for increased HBP flux for $Kras^{G12D}$-induced tumorigenesis and tumor maintenance had not yet been explored. Using complimentary genetic and pharmacologic methods *in vitro* and *in vivo*, we showed for the first time that the HBP was required for $Kras^{G12D}$ -induced lung tumorigenesis and lung tumor maintenance. We further established that O-GlcNAcylation was not only required for $Kras^{G12D}$-induced lung tumorigenesis and tumor maintenance *in vitro* and *in vivo*, but that O-GlcNAcylation was also sufficient to accelerate $Kras^{G12D}$-induced lung tumorigenesis.

As HBP flux is central to many glycosylation events, EMT-driven changes in the HBP can potentially impact other glycan classes, such as the N-linked and O-linked glycoproteins. Many N-linked and O-linked glycoproteins are large proteins, such as mucin, which are involved in supporting cell-cell and cell-extracellular matrix (ECM) interactions, and can also promote cancer cell growth, survival and tumor progression. Although our data showed no significant changes in the level of N-linked glycosylations with overexpression of *SNAI1* or *Twist1* with a concanavalin A (ConA) blot, this technique only recognizes the core of the N-linked glycans. The HBP can also impact N-linked glycan branching and many other glycosylation pathways, therefore the impact of the EMT-HBP axis on other glycans will need to be more fully explored in future studies. This EMT-HBP axis may also be broadly important in other types of lung cancer as we have shown previously that *TWIST1* was found to be overexpressed in a majority of human lung cancer samples including not only adenocarcinoma, but also other major lung cancer histologies such as squamous cell carcinoma.

Taken together, our new study demonstrated that EMT driven HBP-O-GlcNAcylation contributes to $Kras^{G12D}$-induced lung tumorigenesis and tumor progression. Thus, inhibition of the HBP and O-GlcNAcylation can impact lung cancer cell death and senescence suggesting the HBP is a novel targetable pathway to indirectly target EMT and *KRAS* mutant driven lung cancers.

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