to be significantly overexpressed, suggesting that OIP5-AS1 may negatively regulate its sense counterpart. In addition, we find that expression of both OIP5 and OIP5-AS1 are significantly associated with 5-year survival. These findings suggest that deregulation of OIP5 through its antisense RNA may represent a novel mechanism regulating tumor phenotypes in NSCLC.

ART1, an extracellular ADP-ribosyltransferase, is over-expressed in non-small cell lung cancer and facilitates cancer cell survival by immune-mediated mechanisms

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Cell surface mono-ADP ribosyltransferases (ARTs) transfer the ADP-ribose moiety from NAD+ to amino acid residues on target proteins and post-translationally regulate their function. Most mammalian mono-ARTs, including ADP-ribosyltransferase 1 (ART1) reside on the cell surface, however the scope of extracellular mono-ADP-ribosylation is largely unknown. It has been suggested that epithelial cells in the injured or inflamed lung may overexpress ART1 as a mechanism of cell survival to protect against cell clearance by inflammatory cells. We hypothesize that ART1 expression is cytoprotective to lung cancer cells and facilitates metastatic growth.

We found ART1 to be expressed in multiple human NSCLC cell lines of distinct driver mutation status by RT-PCR, western blots, and immunofluorescent staining. Using biobanked human materials, we also found evidence of ART1 expression in human NSCLC tumors by whole tumor RT-PCR, immunofluorescence, and immunohistochemistry. Compared to matched adjacent normal lung (n=40), by RT-PCR there is over a 2-fold increase (p=0.01) in median tumor expression of ART1, suggesting a role in tumorigenesis or tumor progression. We subsequently stained a tissue microarray containing 184 cases of predominantly (74%) stage I NSCLC to determine the prevalence of NSCLC tumors staining positive for ART1. ART1 staining was moderate or strong in 83% of adenocarcinomas (n=145) and in 45% of squamous cell cancers (n=39, p<0.001).

We next used murine cell lines derived from inducible KRASG12D/+/p53-/- mice and an in vivo model to determine the effect of ART1 expression on metastatic outgrowth. In a tail vein injection model in immunocompetent mice, we noted a highly significant decrease in metastasis in the ART1-knockdown cell line (sh175) compared to the parent KP1 line. To determine whether the immune protective effect mediated by ART1 may be T cell dependent, we implanted flank tumors in immunocompetent (B6) and lymphocyte-depleted nude mice. Despite slightly faster in vitro rates of growth, sh175KP1 cells lacking ART1 expression were largely unable to form tumors when injected into the flanks of immunocompetent B6 mice. Only 1 of 5 immunocompetent mice injected with sh175KP1 cells developed a tumor, compared to 5 of 5 mice injected with KP1 control cells. However, in lymphocyte-depleted nude mice, there was robust flank tumor growth with both cell lines. Because the sh175KP1 cells lacking ART1 only had a disadvantage to growth in mice with lymphocytes, we can infer that ART1 expression may have a strong immunomodulating effect on that cell population. We also evaluated the effect of ART1 expression on neutrophil cytotoxicity. At a neutrophil:tumor cell ratio of 20:1, the knockdown cell line sh175KP1 lacking ART1 expression is more sensitive to neutrophil-induced apoptosis in the co-culture assay (87% vs. 56% Annexin V positive, p=0.05). Importantly, chemical inhibition of mono-ADP-ribosylation in the parent KP1 cell line with two well established inhibitors, novobiocin and meta-iodobenzylguanidine, facilitated neutrophil-induced apoptosis, implying that the enzymatic activity of ART1 is critical to the phenotype.

We provide evidence that ART1 is overexpressed in NSCLC. ADP-ribosylation may serve as a defense against immune-mediated cytotoxicity. Cells without ART1 expression are more sensitive to cytotoxicity induced by immune cells and these cells have a markedly decreased capacity to grow in the lungs in an immunocompetent metastatic model. Because ART1 is an extracellular enzymatic target, it is expected to be highly druggable and susceptible to therapeutic intervention. We envision using targeted inhibition of ADP-ribosylation in ART1-overexpressing tumors to facilitate immune-mediated destruction of established cancers or of micrometastatic disease.

Routine molecular testing of resected early-stage lung adenocarcinoma with targeted next-generation sequencing demonstrates a high rate of actionable mutations

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Introduction: Molecular testing is routinely performed in patients with metastatic non-small cell lung cancer. As the