Published OnlineFirst May 22, 2017; DOI: 10.1158/1078-0432.CCR-16-2540

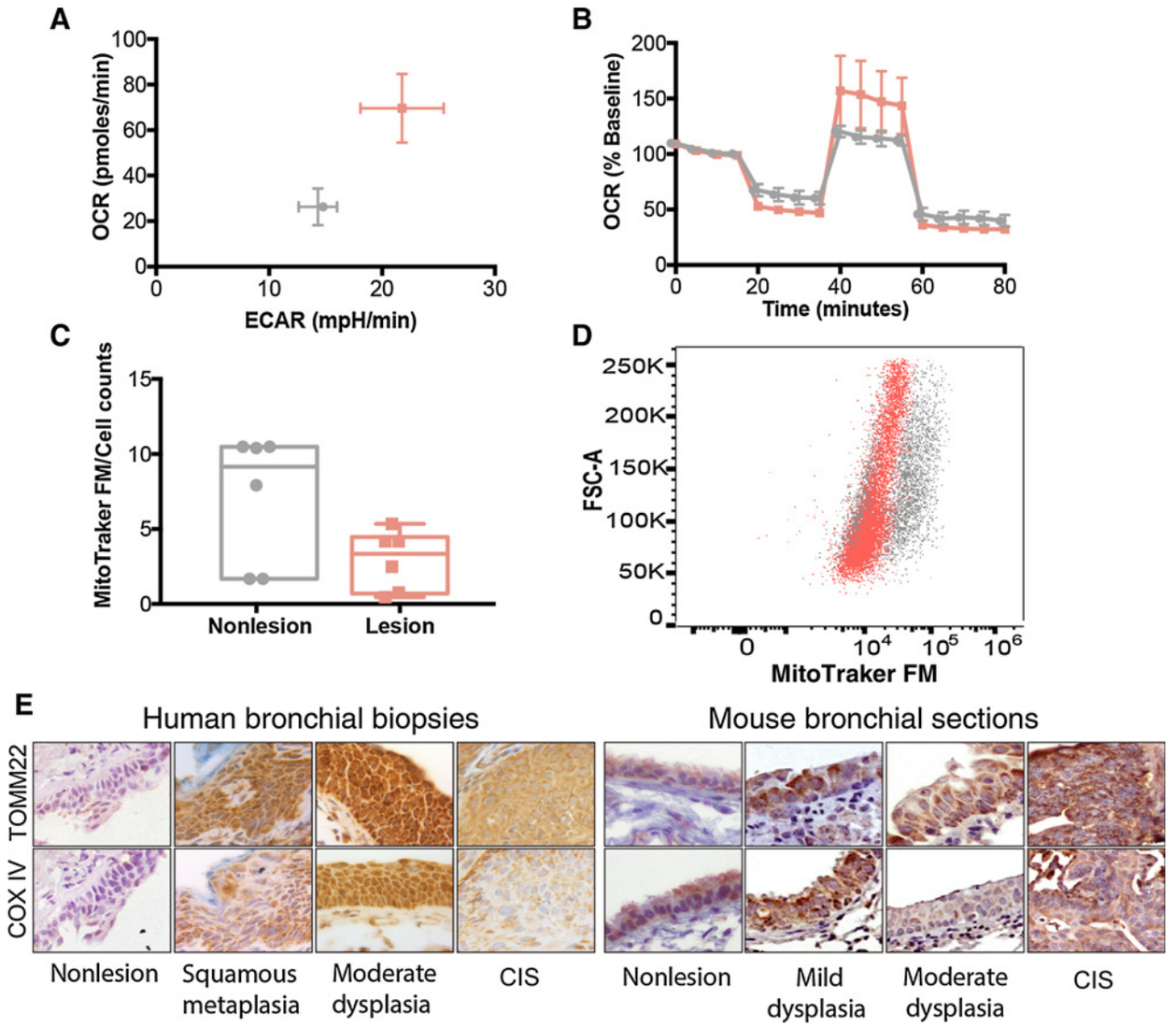

Figure 3.

OXPHOS upregulation in premalignant lesion biopsies. **A,** The mean baseline OCR/ECAR ratio measured in human bronchial biopsy cultures from PMLs (pink, $n = 6$) was 2.5-fold higher than the biopsies of normal airway epithelium (gray, $n = 6$; $P = 0.035$). **B,** Bioenergetic studies testing mitochondrial function demonstrate PMLs (red) have a significantly (~1.5-fold) higher maximal respiration ($P = 0.022$). Error bars (**A** and **B**), SEM. **C** and **D,** Mitochondrial enumeration by FACS analysis of MitoTraker GFP suggests increased OCR is not reliant on increased mitochondria as the difference in GFP per cell was not significant ($P = 0.150$). **E,** Representative images of TOMM22 and COX IV staining in which expression of both proteins is increased in low and moderate dysplastic lesions in both human and NTCU-mouse PMLs (magnification, ×400).

(which can be difficult to observe under white light) and thus improve identification of high-risk smokers that should be targeted for aggressive lung cancer screening programs. In addition, the biomarker may offer wider clinical utility in early intervention trials by serving as an intermediate endpoint of efficacy (beyond Ki-67 staining for proliferation, and changes in biopsy histology). Toward this goal, we demonstrated that the change in biomarker scores over time reflects contemporaneous regressive or progressive/stable disease (AUC = 0.75). This result suggests that the airway field of injury in the presence of PMLs is dynamic and that capturing the gene expression longitudinally may allow for further stratification of high-risk subjects. The potential clinical utility of the biomarker is further supported by recent work demonstrating a significant association between the development of incident lung SCC and the frequency of sites that persist or progress to high-grade dysplasia (24).

Further development and testing in a larger cohort is needed to confirm the biomarker's performance, utility, and ability to predict future PML progression or regression. In addition, longitudinal and spatial sampling would provide a greater understanding of the dynamic relationship between the normal epithelium and the PMLs as they regress or progress to SCC. Longitudinal studies would allow for more accurate

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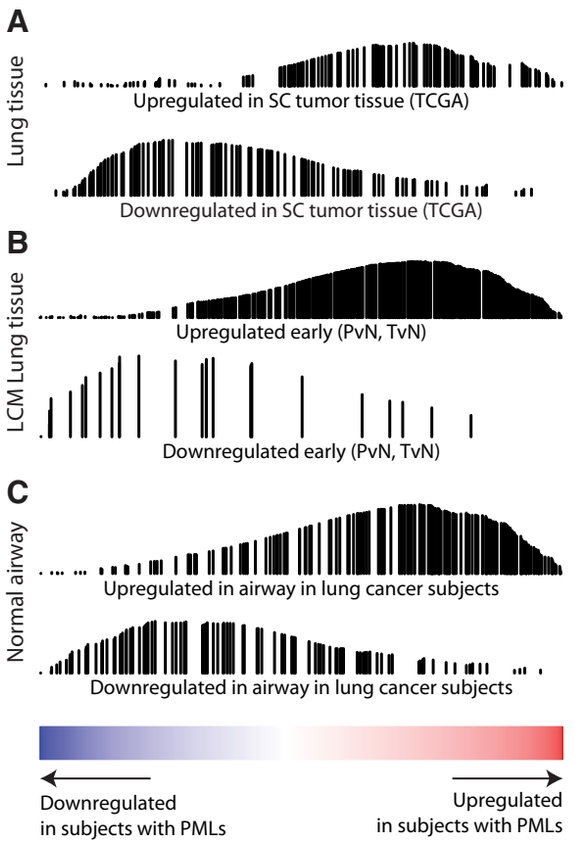

Figure 4.

PML-associated gene expression alterations in the airway field are concordant with SCC-related datasets. Genes are ranked by their differential expression in subjects with and without PMLs (*x*-axis, genes up-regulated and down-regulated in subjects with PMLs are red and blue, respectively). The SCC-related gene signatures were significantly and concordantly enriched among PML-associated gene expression changes by GSEA. The black vertical lines represent the position of genes in the ranked list (*x*-axis) and the height corresponds to the magnitude of the running enrichment score from GSEA (*y*-axis). **A,** Top differentially expressed genes from analysis of TCGA RNA-Seq data comparing lung SCC and matched adjacent normal tumor tissue. **B,** Ooi and colleagues gene sets for early gene expression changes defined by genes altered between premalignant and normal tissue and between tumor and normal tissue ($P < 0.05$) using laser capture microdissected (LCM) epithelium from the margins of resected SCC tumors. **C,** Top differentially expressed genes from analysis of cytologically normal bronchial epithelial cells from smokers with and without lung cancer (GSE4115).

characterization of the time intervals needed to observe gene expression dynamics both in the PMLs and in the airway field of injury. Spatial sampling throughout the respiratory tract, including the more accessible nasal airway that shares the tobacco-related injury with the bronchial airways (54), would allow for evaluation of the impact of distance between the PMLs and the brushing site, the range of PML histologies, and the multiplicity of PMLs that can be present simultaneously in a patient and influence the PML-associated airway field.

In light of these challenges and opportunities for future work, we have comprehensively profiled gene expression changes in airway epithelial cells in the presence of PMLs that suggest great clinical utility. Moving therapeutics and detection strategies toward an earlier stage in the disease process via molecular characterization of premalignant disease holds great promise (55, 56), and this study represents an important step toward a precision medicine approach to lung cancer prevention.

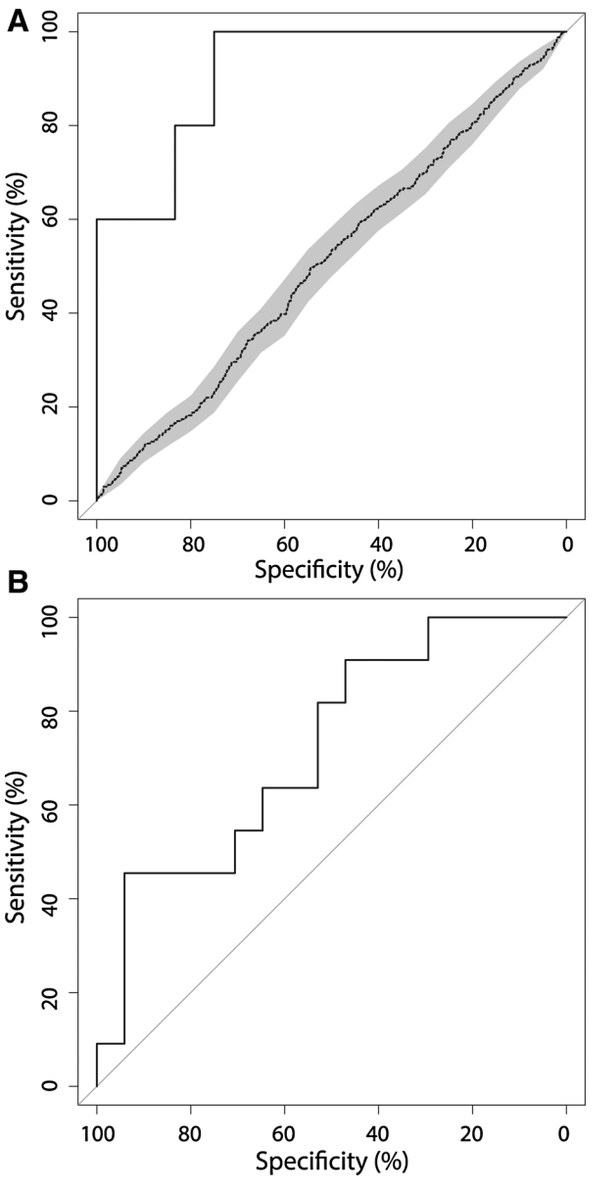

Figure 5.

Performance of an airway biomarker in detecting the presence and progression of premalignant lesions. The ROC curves demonstrate the biomarker performance. **A,** ROC curve (AUC = 0.92) showing the performance of the biomarker detecting presence of PMLs in the validation samples ($n = 17$), solid line. Shuffling of class labels ($n = 100$ permutations) produced an average ROC curve (dotted line) with a significantly lower AUC ($P < 0.001$) and a narrow confidence interval (shaded area). **B,** ROC curve (AUC=0.75) showing the performance of biomarker score differences over time detecting PML regression or stability/progression.

Disclosure of Potential Conflicts of Interest

J. Beane reports receiving commercial research grants from Janssen Pharmaceuticals. A.M. Tassinari reports receiving a commercial research grant from Janssen Pharmaceuticals. M.E. Lenburg reports receiving commercial research grants from Janssen Research and Development, Inc. and is a consultant/advisory board member for Veracyte. A.E. Spira reports receiving commercial research grants from Janssen Pharmaceuticals and is a consultant/advisory board member for Janssen Pharmaceuticals and Veracyte. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.A. Mazzilli, G. Liu, H. Liu, A. Dy Buncio, S.S. Dhillon, M.E. Reid, S. Lam, A.E. Spira

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Beane, S.A. Mazzilli, A.M. Tassinari, H. Liu, A. Dy Buncio, M.E. Lenburg, M.E. Reid

Writing, review, and/or revision of the manuscript: J. Beane, S.A. Mazzilli, A.M. Tassinari, S.S. Dhillon, S.J. Platero, M.E. Lenburg, M.E. Reid, S. Lam, A.E. Spira

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Acknowledgments

We thank Daniel Merrick (University of Colorado, Denver) and the Denver SPORE for facilitating the acquisition of premalignant primary cultures used in this study. We also thank Candace Johnson (Roswell Park Cancer Institute) for providing NTCU-mouse tissue for analysis in this study as well as Mary Beth Pine for providing clinical data for the human bronchial brushes from the Roswell Park Cancer Institute.

Grant Support

This work was supported by LUNGevity Foundation grant #2012-01 (to J. Beane) and Janssen Pharmaceutical Research and Development, L.L.C. Sponsorship (to A. Spira).

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Received October 14, 2016; revised March 23, 2017; accepted May 17, 2017; published OnlineFirst May 22, 2017.

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Published OnlineFirst May 22, 2017; DOI: 10.1158/1078-0432.CCR-16-2540

Clinical Cancer Research

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Clin Cancer Res 2017;23:5091-5100. Published OnlineFirst May 22, 2017.