Genomic characterization of a chemically-induced murine lung squamous cell carcinoma model

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Abstract

INTRODUCTION: Model systems for the study of cancer are each fraught with limitations in recapitulating human disease. Among them, carcinogen-induced models in animals have the capacity to recreate disease states which closely resemble the characteristics of similar diseases in human; however, a reproducible carcinogen-induced model of murine lung squamous cell carcinoma has been difficult to establish, and the molecular characteristics of these tumors has not been sufficiently compared with the human counterparts. We hypothesize that N-nitrosotris-(2-chloroethyl)urea (NTCU) induced murine lung squamous cell carcinoma (LSCC) represents a reproducible model with molecular changes that more ideally represent human disease.

METHODS: A total of 5 mice per group were either treated with vehicle or with the LSCC inducing carcinogen NTCU over the course of 20-24 weeks. Tumor development was monitored via small animal MRI scan of the lungs and sacrifice of the animals was performed at the designated timepoint. Areas of normal lung tissue were captured from vehicle-treated animals and areas representing either dysplasia or squamous cell carcinoma were captured from NTCU-treated animal lungs using laser capture microdissection (LCM). RNAseq and DNAseq were then performed utilizing the LCM tissue sections. In addition, a cell line was isolated and propagated from one of the tumors. Various studies were performed using the RNAseq data from LCM tissues as compared to simultaneously collected control tissue. In order to compare with human disease, data was analyzed against human expression data from the Cancer Genome Atlas (TCGA) and other publicly available datasets.

RESULTS: Gene expression results from RNAseq experiments showed that dysplasia and SCC samples segregated from normal tissue based on principal component analysis. Hierarchical clustering of expression data revealed a distinct expression pattern separating dysplasia and squamous cell carcinoma tissues from normal. Additional analyses showed significant overlap of the expression changes observed in the NTCU-model of murine LSCC and human LSCC.

CONCLUSION: Treatment of mice with NTCU reliably induces lung dysplasia and SCC. The molecular changes of the tumors that develop share significant similarity to human LSCC as compared through TCGA data. Murine NTCU models of LSCC are adequate surrogates for human disease.

Introduction

- The study of cancer and other disease states relies heavily on animal models of disease; however, a known limitation of animal study is failure to recapitulate the human phenotype with high fidelity[1]
- Current systems include patient-derived xenograft models (PDX), genetically engineered mouse models (GEMM), syngeneic models, or chemically (carcinogen) induced models
- Each model bears its own limitations in studying human disease
- PDX models require an immunosuppressed host animal which fail to capture complexities of the immune response on tumor progression, cell lines undergo drift over time and may be dissimilar from the original tumors
- GEMM models lack the mutational diversity of human tumors[2]
- Syngeneic models typically require tumor induction in non-anatomic sites of disease (such as orthotopic)
- Carcinogen-induced models offer the greatest opportunity for modeling of a human disease state, but are limited by reproducibility and characterization of the tumors that develop[3]
- We hypothesize that exposure to N-nitrosotris-(2-chloroethyl)urea (NTCU) will lead to murine lung dysplasia and LSCC that is genetically similar to human LSCC and thus represents a more ideal model system

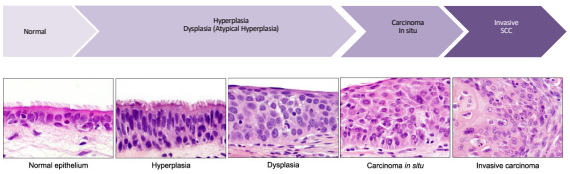


Figure 1. Development of lung squamous cell carcinoma follows a known progression of cellular and molecular changes

Methods

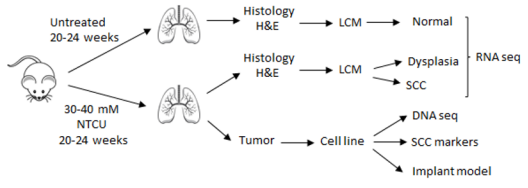
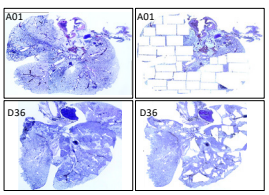


Figure 2. Flow diagram of animal treatment schedule as well as the strategy for sample collection/processing used in genomic characterization. Five samples were generated in each group of normal, dysplasia, and SCC. LCM – laser capture microdissection



Mouse ID	Tissue sample	NTCU dose (mM)	Sex	Sample date
A01	Normal	0	F	8/16/2016
A02	Normal	0	F	8/10/2016
A08	Normal	0	M	8/16/2016
A07	Normal	0	M	8/10/2016
A06	Normal	0	M	8/10/2016
D36	Dysplasia	30	F	8/16/2016
D36	SCC	30	F	8/16/2016
D39	Dysplasia	30	F	8/16/2016
D39	SCC	30	F	8/16/2016
D42	Dysplasia	30	F	8/17/2016
D42	SCC	30	F	8/17/2016
D44	Dysplasia	30	M	8/17/2016
D44	SCC	30	M	8/17/2016
E57	Dysplasia	40	F	8/17/2016
E57	SCC	40	F	8/17/2016

Figure 3. Example laser capture microdissection of lung tissue from control animals (designated ID AXX) and NTCU treated animals (designated ID DXX or EXX)

Results

Dysplasia and LSCC tissue samples are genetically dissimilar from normal tissue as identified on principal component analysis

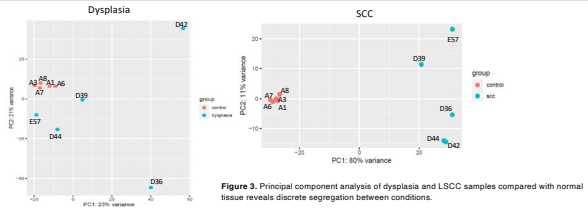
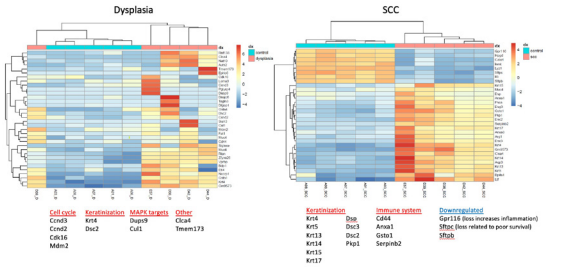
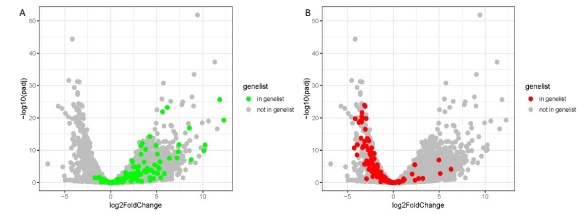


Figure 3. Principal component analysis of dysplasia and LSCC samples compared with normal tissue reveals discrete segregation between conditions.

Hierarchical clustering based on RNAseq expression data reveals common patterns of differential expression among pathological states compared to normal



Differential gene expression in mouse LSCC induced by NTCU has great similarity to expression in human LSCC



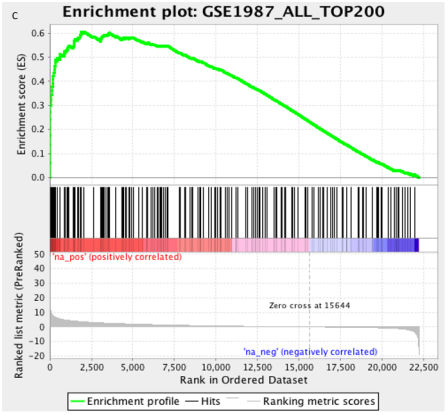


Figure 4. Differential gene expression analysis of lung squamous cell carcinoma samples show significant overlap of over- (A) and under-expressed (B) genes in the NTCU-induced mouse tumors and expression changes noted within TCGA data. Gene Set Enrichment Analysis (GSEA)[4,5] of genes over- and under-expressed (C) in murine lung squamous cell carcinoma showed significant correlation with human expression alterations.

Discussion

- Topical treatment with NTCU induces murine lung dysplasia and LSCC in a reliable fashion by 20-24 weeks after initiating treatment
- Comparison of dysplasia and LSCC samples to normal tissues reveals a distinct set of molecular changes which separate these disease states from normal tissues in treated mice
- Expression changes in murine lung dysplasia and SCC induced by NTCU show differences from normal tissue in pathways related to cell cycle, keratinization, MAPK targets, and immune system interaction
- Evaluation of specific up- and down-regulated genes showed similarity to human LSCC which was verified by Gene Set Enrichment Analysis
- NTCU-induced murine lung SCC models are promising for further study and appear genetically similar to human LSCC

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