Discriminating Spontaneous Tumors from Exposure-Induced Tumors in the A/J Mouse Lung Cancer Model

Yang Xiang, Florian Martin*, Karsta Luettich, Keyur Trivedi, Emmanuel Guedj, Ee-Tsin Wong, Julia Hoeng, Manuel C. Peitsch

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchatel, Switzerland
* Corresponding author: Florian.Martin@pmi.com.

Introduction and Objectives

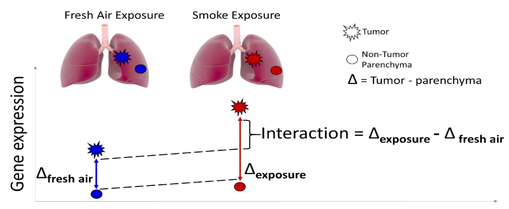
The A/J mouse model is highly susceptible to chemical lung tumor induction and has been widely used as a screening model in carcinogenicity testing and chemoprevention studies. Although cigarette smoke (CS) exposure induces tumors in the lungs, non-exposed A/J mice will also develop lung tumors spontaneously as they age. This raises the question whether the exposure-induced tumors are of a similar type to spontaneous tumors, irrespective of the overall exposure effect. As exposed mice may exhibit both exposure-induced and spontaneous tumors, this leads to a one-class problem, as only spontaneous tumors arising from non-exposed animals are unequivocally defined.

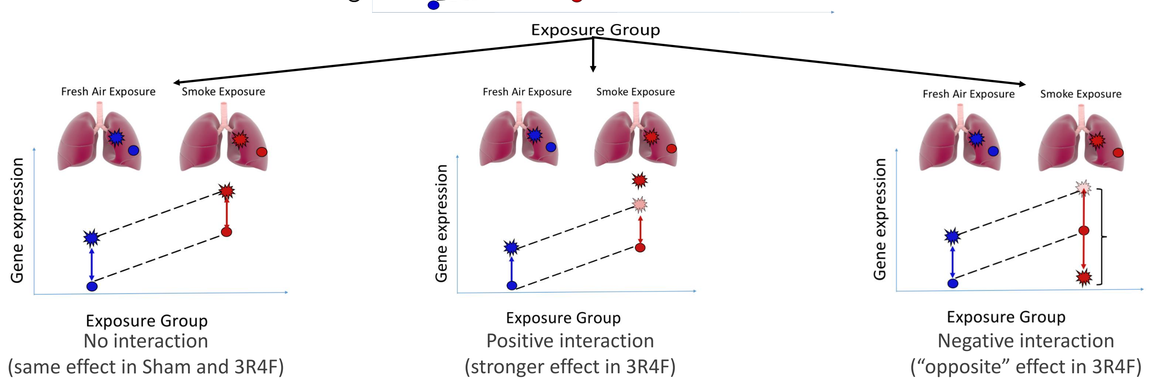
We would like to develop a gene signature and a one-class classifier to examine the potential differences between tumors developing in exposed vs. unexposed A/J mice. The gene expression pattern should be specific to the tumor state, as opposed to gene expression dysregulation by the exposure itself (e.g., xenobiotic response genes).

Material & Methods

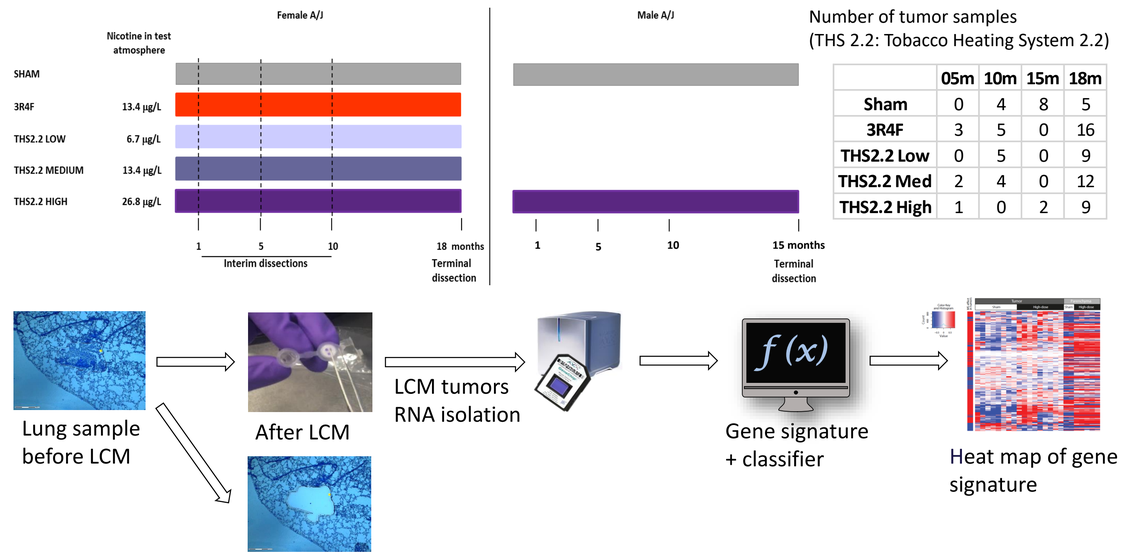
A. Genes having a specific behavior in spontaneous tumors vs. 3R4F reference cigarette (University of Kentucky, Lexington, KY, USA) CS-exposed tumors were ranked in an independent study [1].

	Number of Mice	
Tissue	Fresh Air Exposure	Smoke Exposure
Parenchyma	**2**	**5**
Tumor	**8**	**9**





B. Experimental design and illustration of workflow in current study



Number of tumor samples
(THS 2.2: Tobacco Heating System 2.2)

	05m	10m	15m	18m
Sham	0	4	8	5
3R4F	3	5	0	16
THS2.2 Low	0	5	0	9
THS2.2 Med	2	4	0	12
THS2.2 High	1	0	2	9

C. Flowchart of building gene signature and one-class classifier

- Tumors from 3R4F cigarette smoke exposed mice
- Spontaneous tumors from fresh air (Sham) exposed mice
- New tumor samples

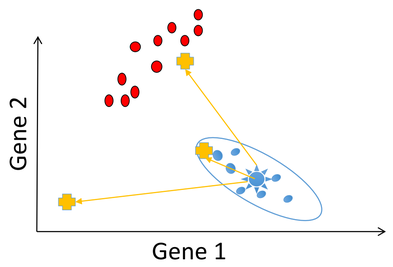


Illustration of one-class classifier

Gene signature: The signature is composed of the first 13 genes with the biggest **absolute interaction value** (> 4.8) in the **previous study** [1], as follows: Scgb3a1, Iglv1, Ighv1-14, Bex1, Ighg3, Chia1, Ighm, Ighg2b, Iglc1, Saa3, Acoxl, Itih4, Ighg1.

One-class classifier

Input: matrix of gene expression values of spontaneous tumors x_i (i=1, …, N), and new sample x_j. Here N is the number of spontaneous tumors.

The probability distribution of the spontaneous tumor (blue point) is described by a multivariate Gaussian distribution, as follows:

$$y_i = \frac{1}{\sqrt{2\pi|\Sigma|}} e^{-\frac{1}{2}(x_i-u)^T\Sigma^{-1}(x_i-u)}$$

where i is the index of sample i, Σ is the covariance matrix, and u is the mean of this multivariate Gaussian distribution.

The Mahalanobis distance [2] method was used as a one-class classifier for new sample j (orange point), as follows:

$$D_j^2 = (x_j - u)^T \Sigma^{-1}(x_j - u) \sim \chi^2$$

$$\text{P-value} = P_{\chi^2}(D_j^2)$$

Output: If the p-value was above 0.05, sample j was believed to belong to the Sham group and, if not, it would be classified as non-Sham tumor.

Figure 1. Panel A: *an interaction model was used to extract genes having a specific behavior in spontaneous tumors vs. exposed tumors in an independent study [1]. There are two different tissue types - parenchyma (○) and tumor (☼) - in the lungs of fresh air-exposed (Sham) and CS-exposed mice. Colored symbols illustrate various expression levels of a given gene. The interaction term is depicted, reflecting the changes in gene expression that were different in tumor compared to the surrounding parenchyma tissues following exposure. In other words, the genes with significant interaction are those whose levels were differentially affected between the two tissue types upon exposure. Three type of interactions - no interaction, positive interaction, and negative interaction - are illustrated in the bottom part of panel A.* ***Panel B:*** *experimental design and illustration of workflow in current study [3]. To assess the impact of lifetime exposure to Tobacco Heating System (THS) 2.2 aerosol compared with that of 3R4F CS on development of emphysema and on lung tumor incidence and multiplicity, female A/J mice were exposed to fresh air (Sham), 3R4F mainstream CS corresponding to 13.4 µg nicotine/L, or THS 2.2 aerosol equivalent to nicotine concentrations of 6.7 (Low), 13.4 (Medium), or 26.8 µg/L (High) [3]. Mice were exposed for 6 hours per day, 5 days per week, for up to 18 months following OECD Test Guideline 453 for combined chronic toxicity/carcinogenicity studies. In addition, two groups of male A/J mice were also exposed to fresh air (Sham) and THS 2.2 aerosol at 26.8 µg nicotine/L.* ***Panel C:*** *The flowchart of building gene signature and one-class classifier. The Mahalanobis distance was used as a one-class classifier, and the gene signature was built based on a previous study [1].*

Results

- The accuracy in 10 times 10-fold cross-validation is 75%, which means that the distribution of the Sham group was not over-fitted.
- Lung tumors in 3R4F CS-exposed mice were significantly different from those in air-exposed animals (p-value < 0.001), indicating different type of tumors in 3R4F CS-exposed mice.
- Lung tumors from THS 2.2 aerosol-exposed mice were not significantly different from those in Sham mice but were significantly different from those in 3R4F CS-exposed mice. Tumors from THS 2.2 aerosol-exposed mice resemble those from air-exposed mice.
- The classification of the tumors from male A/J mice exposed to THS 2.2 aerosol was not as significant as that for the corresponding female study group, because the number of tumors in the male THS 2.2 aerosol-exposed mice was small (N=2).

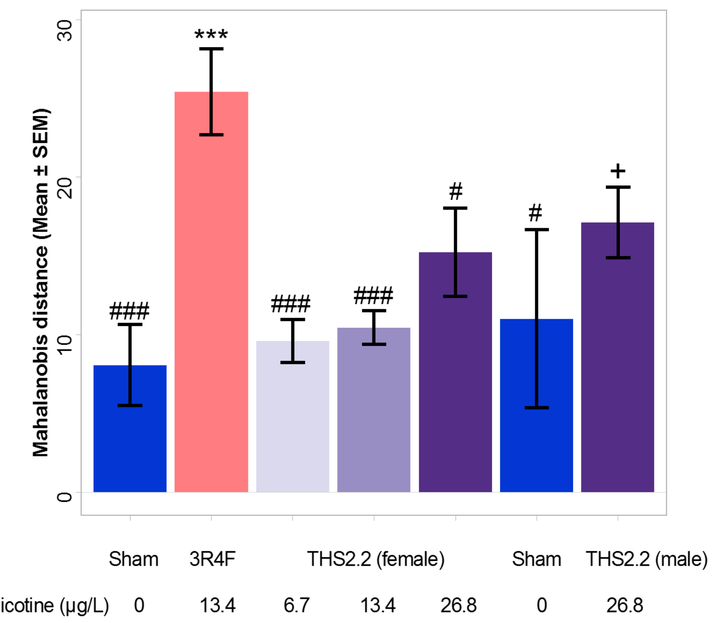


Figure 2. Estimates of similarity between lung tumors based on Mahalanobis distance*. As an estimate of similarity between lung tumors, the Mahalanobis distance between lung tumors in Sham animals and those in each exposure condition was calculated based on a 13-gene signature derived from an interaction analysis of gene expression data from a previous A/J study (E-MTAB-1871) [1]. Results are presented as mean ± standard error of the mean (SEM). Significant differences between exposure groups and Sham are represented by * (p-value < 0.05), ** (p-value < 0.01), and *** (p-value < 0.001); significant differences between THS 2.2 aerosol and 3R4F CS exposure groups are represented by # (p-value < 0.05), ## (p-value < 0.01), and ### (p-value < 0.001). + indicates that there were only two tumor samples in this study group.*

- The majority of lung tumors in THS 2.2 aerosol-exposed mice were similar to the lung tumors in Sham mice, but different from those in 3R4F CS-exposed mice, as shown in Figure 3.

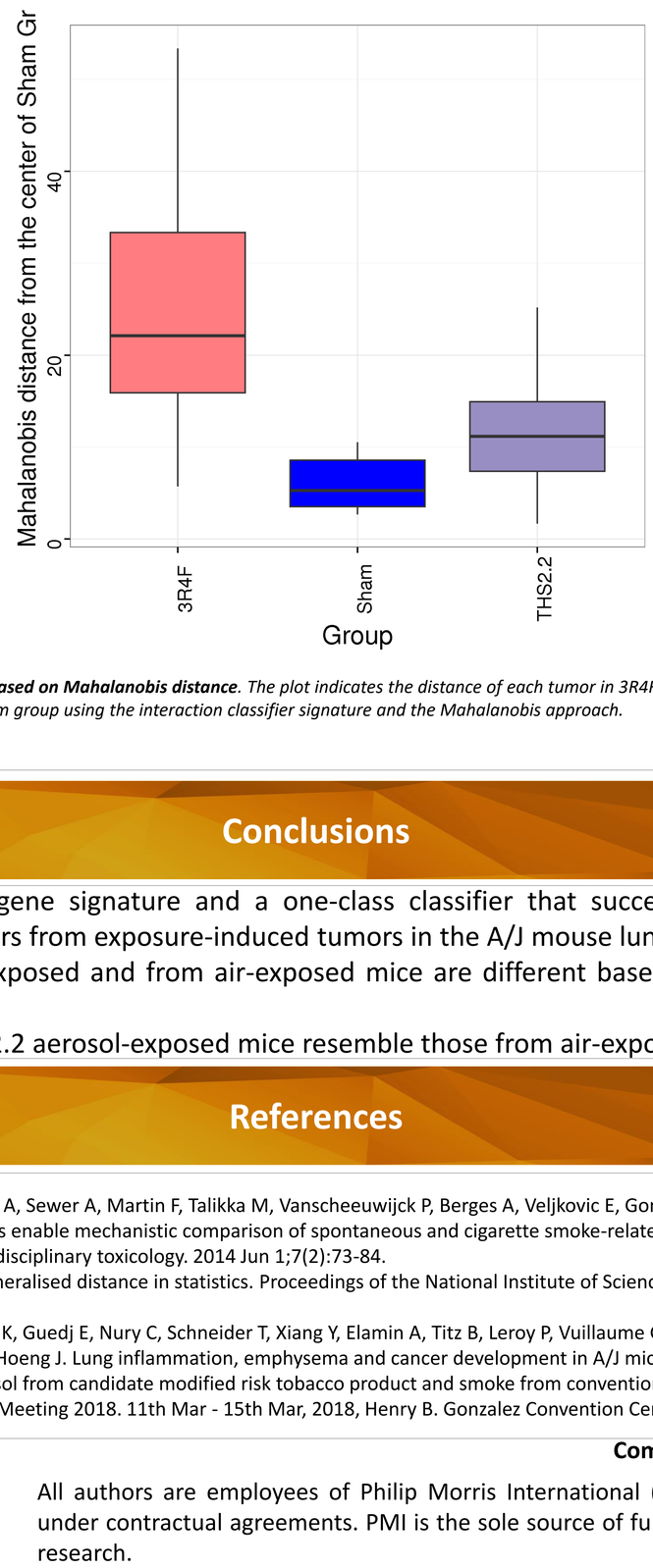


Figure 3. Tumor classification based on Mahalanobis distance*. The plot indicates the distance of each tumor in 3R4F CS- or THS 2.2 aerosol-exposed mice from the center of the Sham group using the interaction classifier signature and the Mahalanobis approach.*

Conclusions

- We developed a gene signature and a one-class classifier that successfully discriminates spontaneous tumors from exposure-induced tumors in the A/J mouse lung cancer model.
- Tumors from CS-exposed and from air-exposed mice are different based on transcriptomics profiles.
- Tumors from THS 2.2 aerosol-exposed mice resemble those from air-exposed mice.

References

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Competing Financial Interest

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