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Tobacco-specific carcinogens induce hypermethylation, DNA adducts and DNA damage in bladder cancer

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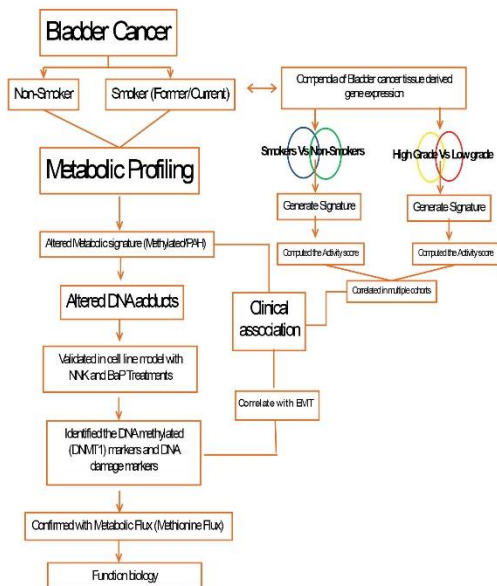
Introduction

Aromatic amines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been strongly implied as carcinogenic for the bladder. NNK may promote tumor metastasis by regulating cell motility by generating DNA adducts and causes DNA damage. Earlier, we identified the methylation-induced enzyme suppression associated with xenobiotic metabolism in bladder tumors. However, the identification of smoke-induced BCa, metabolic signature and its downstream effects are largely understudied. In this study, we have shown higher levels of methylated polycyclic aromatic hydrocarbons, DNA adducts, as well as DNA damage in smokers with BCa. The results suggests a potential causal role of methylation in the accumulation of DNA adducts and DNA damage in carcinogen-induced Bca.

Agilent 6495 LC/TQ was used in this experiment to identify the metabolites in bladder cancer samples.

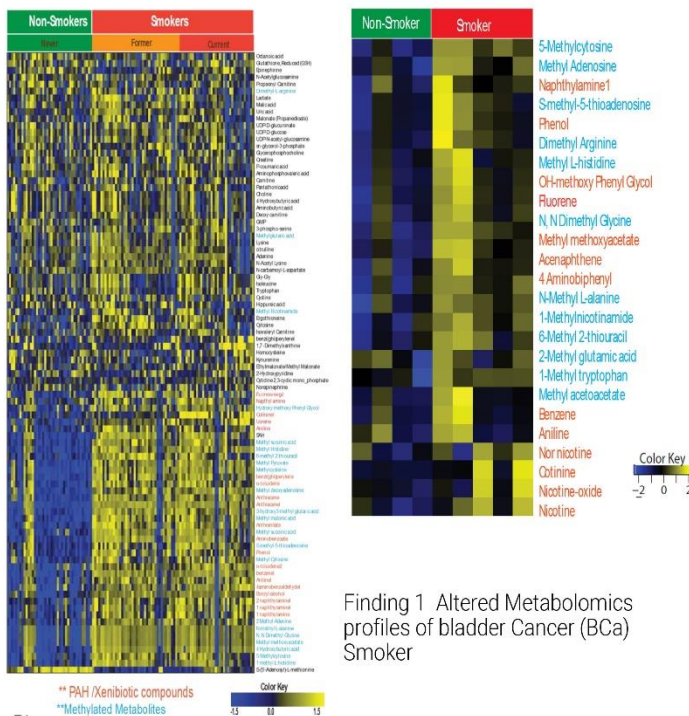
Experimental

Overview of the strategy used to profile and characterize the metabolome of bladder cancer samples from smokers (n=78) and non-smokers (n=41)

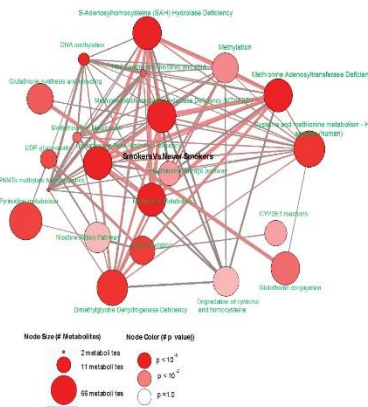


Results and Discussion

Heat map of hierarchical clustering of levels of 90 metabolites detected across 119 bladder cancer samples.



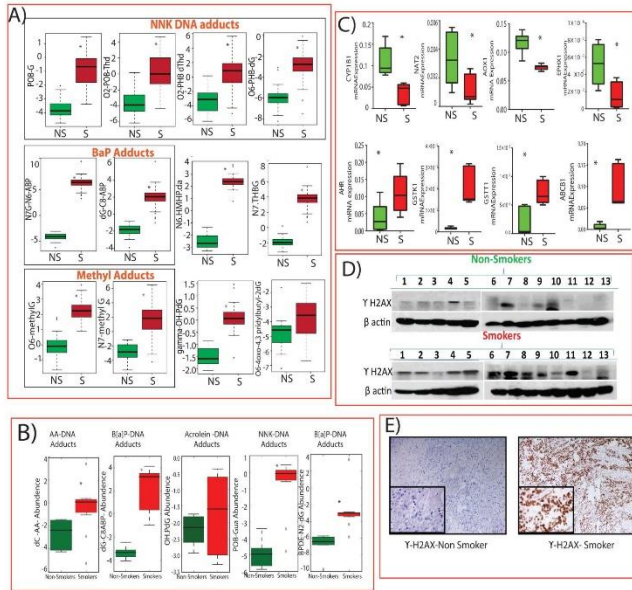
Finding 1 Altered Metabolomics profiles of bladder Cancer (BCa) Smoker



Pathway analysis of the metabolic profiles in our "BCa smoking-associated metabolic signature." The node size is proportional to the number of metabolites in the process or condition. Color represents a statistically significant enrichment (p-value)

Results and Discussion

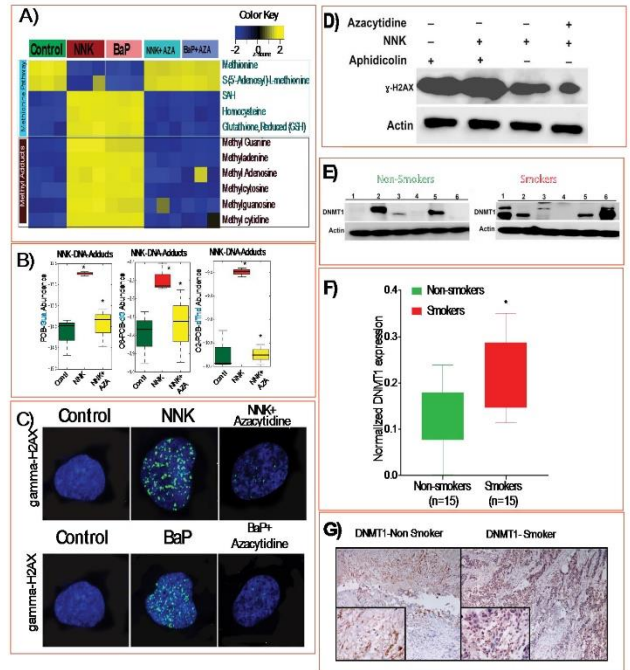
Levels of DNA adducts, Xenobiotic enzymes, DNA damage markers in BCa between smokers and non-smokers



A) Box plots showing higher expression of NNK, BaP, and methyl DNA adducts in BCa from smokers (S; n=15) than in that from non-smokers (NS; n=15). *, p<0.005. B) Box plots showing higher levels of DNA adducts from the urine pellet of smokers (n=5) than non-smokers (n=5). *, p<0.005. C) Box plots showing relative transcript levels for xenobiotic enzymes obtained by real-time PCR of BCa samples from non-smokers (n=6) and smokers (n=6). *, p<0.005. D) Protein levels of Y-H2AX in non-smokers (n=13) and smokers (n=13) with BCa as determined by western blotting. E) Immunohistochemical staining for Y-H2AX expression

Results and Discussion

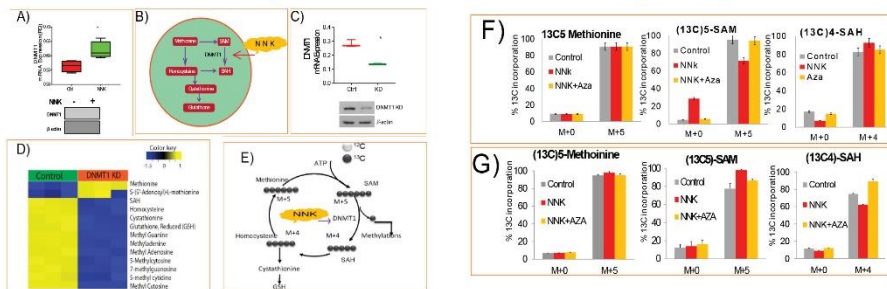
Tobacco-specific carcinogens induced methylation, DNA adducts, DNA damage in BCa smokers show higher DNMT1 expression than non-smokers



A) Box plots showing higher expression of NNK, BaP, and methyl DNA adducts in BCa from smokers (S; n=15) than in that from non-smokers (NS; n=15). *, p<0.005. B) Box plots showing higher levels of DNA adducts from the urine pellet of smokers (n=5) than non-smokers (n=5). *, p<0.005. C) Box plots showing relative transcript levels for xenobiotic enzymes obtained by real-time PCR of BCa samples from non-smokers (n=6) and smokers (n=6). *, p<0.005. D) Protein levels of Y-H2AX in non-smokers (n=13) and smokers (n=13) with BCa as determined by western blotting. E) Immunohistochemical staining for Y-H2AX expression

Results and Discussion

SNNK induce DNMT1 expression and altered methionine pathway in BCa



A) Box plots showing mRNA and protein expression of DNMT1, by real-time PCR and western blot, respectively, in J82 BCa cells treated with or without nitrosamine NNK. B) The role of DNMT1 in the methionine pathway. C) Confirmation of DNMT1 knockdown (KD) by mRNA and protein expression analyses in J82 cells. D) Heat map of metabolites in control and shRNA knockdown (KD) of DNMT1 in J82 cells. E) ¹³C(U) ux in methionine pathway. F) J82 cells were pretreated with NNK or NNK+ AZA prior to the addition of ¹³C(U) Methionine. Cell pellets were collected after ¹³C(U) methionine addition and isotopomeric distribution for indicated metabolites was measured using mass spectroscopy. Individual isotopomers are graphed as a percent of the total pool for the indicated metabolite. (G) Experiments were performed in J82. Data in bar graphs are represented as means \pm SEM. n=3 independent cultures for isotopomeric distributions in F and G. Statistical analysis was performed using a two-tailed Student's t-test ($p < 0.05$)

Conclusions

- Specific metabolic signature associated with BCa Smoker
- Identified DNA adducts, DNA damage in BCa Smoker
- Identified molecular mechanisms of methionine pathway alterations and DNMT1 activation using in vitro bladder cancer models.
- Results illustrate the relevance of hyper methylation, DNA adducts, DNA damage and DNMT1 overexpression in tobacco compounds induced carcinogenesis.

References

- ¹Metabolomic Profiling Reveals Potential Markers and Bioprocesses Altered in Bladder Cancer Progression. *Cancer Res.* 2011 Dec 15;71(24):7376-86
- ²Integrative Pathway Analysis of Metabolic Signature in Bladder Cancer: A Linkage to The Cancer Genome Atlas Project and Prediction of Survival. *J Urol.* 2016 Jun;195(6):1911-9.

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