cells

Supplementary Material

The Effect of NNK, A Tobacco Smoke Carcinogen, on the miRNA and Mismatch DNA Repair Expression Profiles in Lung and Head & Neck Squamous Cancer Cells

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Supplementary Table S1: Human genes analyzed by real-time qPCR, in human lung (NCI) and head and neck (FaDu) cancer cells.

Gene	Detected transcripts	Amplicon length (bp)
hGAPDH	NM_001256799, NM_002046	95
Hs_MSH2	NM_000251	86
Hs_MLH1	NM_000249, NM_001167618-9, NM_001258271-4, XM_005265161,3,4,6	127

Supplementary Table S2: Human miRNAs and small RNA analyzed by real-time qPCR, in human lung (NCI) and head and neck (FaDu) cancer cells.

miRNA (human)	**Target mature miRNA (Sanger Accession)**
miR-21	hsa-miR-21-5p, MIMAT0000076
miR-155	hsa-miR-155-5p, MIMAT0000646
miR-422a	hsa_miR-422a-5p, MIMAT0001339
Syn-hsa-miR-21-5p	HSA-MIR-21-5P, MIMAT0000076
Anti-hsa-miR-21-5p	HSA-MIR-21-5P, MIMAT0000076
Small RNA	**Control**
RNU6-2 (RNU6-6P RNA, U6 small nuclear 6, pseudogene)	Hs_RNU6-2_11

Supplementary Table S3. Transcriptional levels of MMR genes in human lung (NCI) and head and neck (FaDu) cancer cells (by qPCR).

A. NCI.

Target gene/ *hGapdh (ΔΔ^{CT})**	**NCI untreated**	**NCI 1μM**	**NCI 2μM**
hMSH2	2.20E-02	2.61E-03	1.32E-03
hMLH1	1.89E-02	2.69E-03	1.64E-03

* normalization of mRNA levels using *hGapdh*

B. FaDu.

Target gene/ *hGapdh(ΔΔ^{CT})**	**FaDu untreated**	**FaDu 1μM**	**FaDu2μM**
hMSH2	8.49E-02	6.25E-02	1.10E-02
hMLH1	2.87E-01	3.26E-02	2.95E-02

* normalization of mRNA levels using hGapdh

Supplementary Table S4. Expression levels of miRNA specific markers analyzed in human lung (NCI) and head and neck (FaDu) cancer cells (by qPCR).

A. NCI.

Target miRNA/ *RNU6 (*ΔΔ*^{CT})**	**NCI untreated**	**NCI 1μM**	**NCI 2μM**
miR-21	2.86E + 00	3.07E + 00	5.98E + 00
miR-155	9.00E-05	8.20E-04	2.05E-03
miR-422a	1.27E-01	9.46E-02	5.30E-02

* normalization of miRNA levels using RNU6

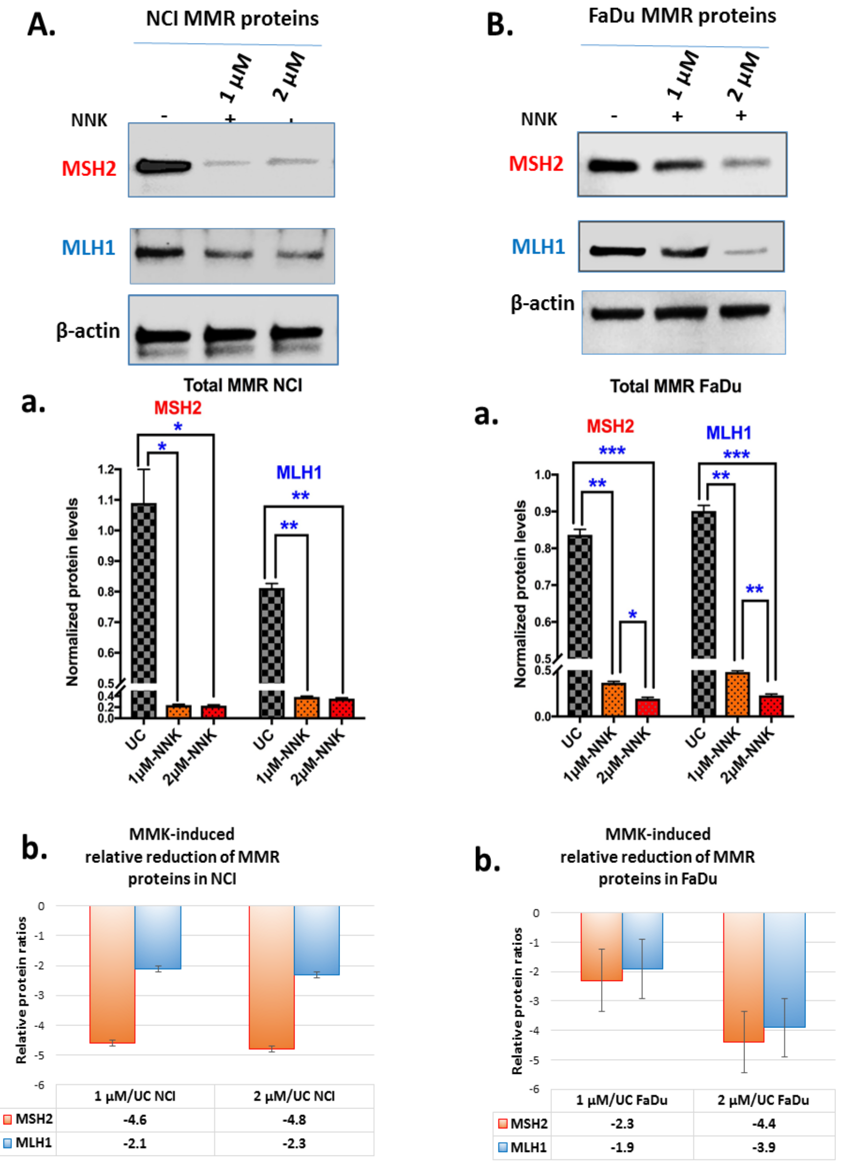
B. FaDu.

Target miRNA/ *RNU6(*ΔΔ*^{CT})**	**FaDu untreated**	**FaDu 1μM**	**FaDu2μM**
miR-21	6.36E-02	5.57E + 00	9.97E + 00
miR-155	8.90E-05	7.74E-02	8.46E-02
miR-422a	3.26E-01	2.56E-02	2.91E-02

* normalization of miRNA levels using RNU6

Supplementary Table S5. Total MSH2 protein level in NCI and FaDu exposed to 1 μM or 2 μM of NNK, with and without miR-21 inhibitor.

	Untreated control	**mimic miR21**	**1 μM NNK**	**miR-inh + 1 μM NNK**	**2 μM NNK**	**miR-inh + 2 μM NNK**
NCI MSH2/β-actin	1.09E + 00	2.68E-01	3.31E-01	5.18E-01	2.93E-01	1.03E + 00
FaDu MSH2/β-actin	8.37E-1	1.70E-01	4.50E-01	5.98E-01	1.92E-01	1.38E + 00



UC: untreated control

Supplementary Figure S1

Supplementary Figure S1. Either low or high dose of NNK reduces the total MMR (MSH2 and MLH1) protein levels in both (**A**) NCI and (**B**) FaDu. Graphs depict MSH2 and MLH1 total levels **(A-a** & **B-a)** and NNK-induced relative reduction of total MMR proteins (expression ratios in NNK-treated vs untreated controls) **(A-b** & **B-b)**, in NCI and FaDu cells, respectively. (β-actin was used to normalize total protein extracts, by western blot analysis; UC: untreated controls). [Paired t-test, * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; cells exposed to 1 μM or 2 μM of NNK compared to untreated controls, by western blot analysis. [Paired t-test, GraphPad Prism 7.0; means(SD) of three independent experiments].