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Gender Specific Differences in RNA Polymerase III Transcription

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Abstract

Background: RNA polymerase (pol) III transcribes a variety of untranslated RNAs responsible for regulating cellular growth and is deregulated in a variety of cancers. In this study, we examined gender differences in RNA pol III transcription *in vitro* and *in vivo*.

Methods: Expression levels of U6 snRNA, tMet, and known modulators of RNA pol III transcription were assayed in male and female derived adenocarcinoma (AC) lung cancer cell lines and male and female C57BL/6J mice using real time quantitative PCR. Methylation status of the U6 snRNA promoter was determined for lung and liver tissue isolated from male and female C57BL/6J mice by digesting genomic DNA with methylation sensitive restriction enzymes and digestion profiles were analyzed by qPCR using primers spanning the U6 promoter.

Results: Here, we demonstrate that RNA pol III transcription is differentially regulated by EGCG in male and female derived AC lung cancer cell lines. Basal RNA pol III transcript levels are significantly different in male and female derived AC lung cancer cell lines. These data prompted an investigation of gender specific differences in RNA pol III transcription *in vivo* in lung and liver tissue. Herein, we report that U6 snRNA RNA pol III transcription is significantly stimulated in the liver tissue of male C57BL/6J mice. Further, the increase in U6 transcription correlates with a significant inhibition in the expression of p53, a negative regulator of RNA pol III transcription, and demethylation of the U6 promoter in the liver tissue of male C57BL/6J mice.

Conclusions: To the best of our knowledge, this is the first study demonstrating gender specific differences in RNA pol III transcription both *in vivo* and *in vitro* and further highlights the need to include both male and female cell lines and animals in experimental design.

Keywords: RNA polymerase III; TFIIIB; C57BL/6J mice; Green tea; EGCG

Introduction

RNA polymerase (pol) III synthesizes small non-coding RNAs involved in the regulation of essential cellular processes, including transcription (7SK), RNA processing (U6 snRNA) and translation (tRNAs) [1]. Aberrant RNA pol III transcription leads to uncontrolled cell growth, a hallmark of many types of cancers [2]. Cells transformed by DNA tumor viruses, or chemical carcinogens, have significant increases in RNA pol III products in ovarian, cervical, esophageal, breast, and lung cancers, as compared to matched normal tissue (reviewed in [2]). Further, RNA pol III transcription is regulated by a variety of oncogenes and tumor suppressors. Tumor suppressors inhibiting RNA pol III transcription include p53 [3-5], PTEN [6,7] BRCA1 [8], RB [4,9,10] and the pocket proteins [11]. Proto-oncogenic products such as c-Myc [4] and CK2 [12] stimulate RNA pol III transcription. Also, host proteins such as MAF1 negatively regulate RNA pol III transcription in response to internal and external cell signals [13-15]. Further, RNA pol III transcription is down regulated by chemopreventive agents such as the polyphenol EGCG in cervical cancer cells [16]. However, regulation of RNA pol III transcription by EGCG has not been demonstrated in other tissue types, nor has general RNA pol III transcription been investigated for gender specific differences.

Herein, we demonstrate that regulation of RNA pol III transcription by EGCG in adenocarcinoma (AC) lung cancer cell lines is gender specific. Also, basal gene external RNA pol III transcript levels are significantly different in male and female derived AC lung cancer cell lines. These observations prompted us to investigate whether general RNA pol III transcription is differentially regulated in males and females *in vivo*. In normal male C57BL/6J mice, gene external RNA polymerase transcription was approximately 50-fold higher than in female C57BL/6J mice. TSP53, a negative regulator of RNA pol III transcription is significantly decreased in the livers of normal male C57BL/6J mice, which may in part explain the observed significant increase in U6 snRNA transcription in male mice. Further, we demonstrate that the U6 snRNA promoter in the liver of male mice is significantly demethylated which may contribute to the 50-fold increase in U6 snRNA transcription in the livers of normal male mice. To the best of our knowledge, this is the first study demonstrating gender and tissue differences in RNA pol III transcription.

Materials and Methods

Cell lines and EGCG treatment

H2347 and A549 cells (ATCC, Rockville, MD) were cultured in RPMI supplemented with FBS (5% v/v), nonessential amino acids (100 mM), L-glutamine (5 mM), streptomycin (100 µg/mL), and penicillin (100 units/mL); all from BioWhittaker, Walkersville, MD. Cells were grown at 37°C in a humidified atmosphere of 95% air and 5% CO_2 as previously described [13,17]. EGCG (Sigma) treatments are as described.

Animals

Animal experiments presented in this study were approved by St. John's University IACUC as part of SJU Protocol number 1741.0. Female and male 5 week old C57BL/6J mice were purchased from Taconic Farms (Germantown, New York). Mice were housed 4 per cage in a temperature-controlled St. John's University Animal Care Facility with alternating 12:12 hr light-dark cycles, with ad libitum access to water and commercial Purina 5001 chow. Mice were numbered with permanent marker identification, and acclimated to animal penthouse for 72 hours prior to start of experiment. Mice were housed for a total of three weeks and monitored daily for signs of stress. Mice were sacrificed by CO_2 inhalation, in accordance with SJU IUCAC approved protocol procedures. The liver and lungs were then harvested for downstream PCR and ELISA applications.

Quantitative PCR (qPCR)

Total RNA was extracted from adenocarcinoma (AC) lung cancer cell lines and animal tissue using the RNeasy total RNA isolation kit (Qiagen), according to the manufacturer's protocol. cDNA was prepared and real time qPCR was performed using diluted cDNA and SsoAdvanced™ Universal SYBR® Green Supermix (BioRad) in the Bio-Rad CFX Connect System. The ΔΔCt method was employed for each gene tested, GAPDH or ARPP expression levels were used for normalization are noted in the figure legend. Statistical analysis was performed using two-tailed students t-test with a 95% confidence interval (Graphpad Prism 3.03); *p<0.05; **p<0.01; ***p<0.001. Sequences of gene specific primers used in PCR reactions are as follows: U6-forward, 5'-GGT CGG GCA GGA AAG AGG GC-3'; U6-reverse, 5'- GCTAAT CTT CTC TGT ATC GTT CC-3'; tRNAiMet-forward, 5'- CTG GGC CCA TAA CCC AGA G-3'; tRNAiMet-reverse, 5'-TGG TAG CAG AGG ATG GTT TC-3'; GAPDH-forward, 5'-TCCACCACCCTGTTGCTGTA-3'; GAPDH-reverse, 5'- ACC ACA GTC CAT GCC ATC AC-3'; ARPP-forward, 5' GCA CTG GGA GTC CAA CTA CTT C-3'; ARPP-reverse, 5'- TGA GGT CCT CCT TGG TGA ACAC-3'; RB-forward, 5'- CCT TGA ACC TGC TTG TCC TCTC-3'; RB-reverse, 5'- CTG AGG CTG CTT GTG TCT CTGT-3'; p53-forward, 5'- CCT CAG CAT CTT ATC CGA GTGG-3'; p53-reverse, 5' –TGG ATG GTG GTA CAG TCA GAGC-3'; CK2-forward, 5'- GGT GAG GAT AGC CAA GGT TCTG-3'; CK2-reverse, 5'- TCA CTG TGG ACA AAG CGT TCCC-3'; BRCA1, MAF1 and MAPK primers were purchased from Qiagen.

Methylation analysis of the U6 snRNA promoter

The U6 promoter was analyzed using MethPrimer [18] to identify potential CpG islands. NEBcutter V2.0 [19] was used to identify methylation sensitive restriction enzyme (MSRE) sites within the U6 promoter sequence. Genomic DNA was isolated from male and female C57BL/6J mice using DNeasy Blood and Tissue Kit (Qiagen) using the manufacturer's protocol. Restriction enzymes used are noted. Restriction digestions were then analyzed by real time PCR using Universal SYBR Green (BioRad) and primers spanning the U6 promoter regions. Results were quantified using the ΔΔCt method and normalized to GAPDH expression levels. Statistical analysis was performed using a two-tailed student's t-test with a 95% confidence interval (Graphpad Prism 3.03); *p<0.05; **p<0.01; ***p<0.001.

Results

EGCG differentially regulates RNA pol III transcription in in male and female derived adenocarcinoma (AC) lung cancer cell lines

Previously, we demonstrated differences in RNA pol III transcription in sex-specific cancer cell lines including the breast, cervical and prostate cell lines [20]. Further, RNA pol III transcription has been demonstrated to be regulated by the purified green tea polyphenol EGCG in the HeLa cervical cancer cell line [16].

However, to the best of our knowledge, this observation has never been reported to be true in other tissue types or whether regulation of RNA pol III is gender specific. Aberrant RNA polymerase III transcripts have been noted in cancers of the breast, lung, ovary, cervix, and esophagus (reviewed in [2]). Further, Lockwood et al., [21] characterized the RNA pol III required BRF2 transcription factor as an oncogene in lung squamous cell carcinomas (SqCC) using integrative genomic analysis. These analyses demonstrated that 40% of SqCC lung cancers have an amplification of chromosome 8 in region 8p12, where BRF2 is localized. Interestingly, the study also demonstrated that 8p12 is deleted in 39% of adenocarcinoma (AC) of the lung, suggesting that in 61% of AC cases, BRF2 DNA is unaltered, but does not provide any evidence whether BRF2-mediated RNA pol III transcription is deregulated in AC lung cancers. Thus, we sought to determine if RNA pol III transcription is regulated by EGCG in AC lung cancer cell lines.

Asynchronous H2347 (female AC) and A549 (male AC) cells were treated with purified 20 uM EGCG for 24 hours. Post treatment, total RNA was isolated for cDNA synthesis. Diluted cDNA was used in two step real time PCR (qPCR) to determine RNA pol III transcript levels in male and female lung AC cells after exposure to EGCG. Our data shows that EGCG significantly inhibited both tMet ($p<0.01$) and U6 snRNA ($p<0.05$) RNA pol III transcription in a female derived AC cancer cell line (Figure 1A). These data agree with our previously published report demonstrating that EGCG inhibited all classes of RNA pol III transcription tested in the HeLa cervical cancer line [16]. Interestingly, EGCG treatment of the male AC lung cancer cells, A549, showed significant stimulation of tMet ($p<0.05$) transcription whereas U6 snRNA transcription was unaffected (Figure 1B). Together, these data suggest that regulation of RNA pol III transcription by EGCG could potentially be driven by gender differences in AC lung cancers.

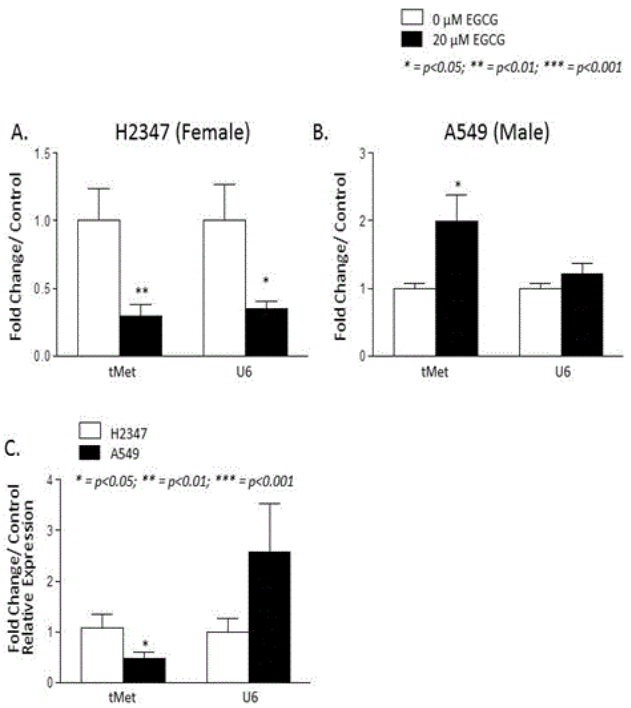

Figure 1: Comparison of RNA polymerase III transcription in male and female derived lung adenocarcinoma cell lines. Female H2347 (A) and male A549 (B) derived AC lung cancer cells were treated with 20 µM EGCG for 24 hours and tMet and U6 snRNA transcription levels determined. Comparison of untreated male A549 and female H2347 RNA pol III transcript levels were performed (C). Total RNA was isolated (A,B,C) from control and treated H2347 and A549 cells for first strand cDNA synthesis. Diluted cDNA was used in quantitative real-time PCR to assay for tMet and U6 snRNA expression using the ΔΔCt method with ARPP expression levels as a reference for normalization. Experiments were performed in triplicate. Statistical analysis performed using one-way ANOVA with Dunnett post-test with a 95% confidence interval (Graphpad Prism 3.03); *p<0.05; **p<0.01; ***p<0.001.

Endogenous RNA pol III transcript levels differ is in male and female derived adenocarcinoma (AC) lung cancer cell lines

Direct comparison of untreated basal tMet and U6 RNA pol III transcription in the male and female derived AC lung cancer cell lines demonstrate that tMet ($p<0.05$) transcription was significantly inhibited in the male derived A549 AC lung cancer cell lines, whereas U6 snRNA appears to be stimulated (Figure 1C), but the observed stimulation is not statistically significant. It has been shown that treatment of mouse epidermal cells with epidermal growth factor (EGF) specifically increases TBP and RNA pol III transcript (tRNA) levels and is inhibited with an epidermal growth factor receptor (EGFR) specific inhibitor [22], suggesting a role for EGFR in the regulation of RNA pol III transcription. Alterations of EGFR have been implicated in the pathogenesis of AC lung cancer [23]. Neither the A549 nor H2347 cell line contain mutations in the EGFR receptor. Interestingly, a comparison of A549 to H2347 shows that the female derived H2347 cell line has a 1.7 fold increase in the relative copy number of EGFR with respect to A549 [23] which may explain the statistically significant increase in tRNA RNA pol III transcription observed in H2347 cells (Figure 1C). Furthermore, the female derived H2347 and male derived A549 cell lines were analyzed for somatic gene mutations using the Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk), database to compare gene mutations present between both cell lines [24]. The Cell Lines Project Resource was analyzed for gene mutations in the cell lines that were classified as having a pathogenic mutation impact and are causally implicated in cancer (cancer gene census). Table 1 represents the genes that met this criterion. KRAS, a well-known lung tumor associated mutation, was found to be mutated in both H2347 and A549 cell lines. In the female derived H2347 cell line, BRCA1 was found to be mutated. We have previously shown that BRCA1 is an inhibitor of RNA pol III transcription, specifically of tRNA and U6 snRNA, and overexpression of BRF1 and BRF2 can alleviate this repression [8]. The ability of EGCG to significantly inhibit U6 snRNA transcription in the female derived H2347 cell lines in comparison to the unaffected U6 snRNA levels in the male derived A549 cell line could be a result of this BRCA1 mutation, which is commonly associated with female specific cancers (Table 1).

Cell Line	Gene Mutations
A549	ATR, CBL, CTNNA1, ERC1, FH, FUS, HIP1, KRAS, POLE, STK11, SUFU, TBL1XR1
H2347	AKAP9, ALK, ARID1B, ATRX, BRCA1, CARD11, CLIP1, CUX1, EP300, EWSR1, EZH2, FAM131B, FGFR4, FOXO4, FOXP1, KDR, KMT2C, KRAS, MECOM, MYH9, NBN, NF1, NRAS, NRG1, NSD1, PHOX2B, PMS1, RANBP2, SND1, SS18, TSC1

Table 1: The Catalogue of Somatic Mutations in Cancer (COSMIC) database (cancer.sanger.ac.uk), specifically the Cell Line Project Resource, was analyzed for mutations in the male derived A549 and female derived H2347 cell lines. The following filters were applied: mutation impact: pathogenic and genes: cancer gene census. Table 1 represents the results of this analysis and lists the genes which are implicated in cancer development and have mutations classified as pathogenic in both A549 and H2347 cell lines.

Together, these data suggest that alterations in RNA pol III transcription may be gender specific in AC lung cancer. These results (Figure 1) are of great significance as the sex of cell lines studied *in vitro* is often ignored, especially in the field of RNA pol III transcription. Our results further support the necessity of the National Institute of Health (NIH) change in grant application policy requiring applicants to report their plans for the balance of male and female cells and animals in preclinical studies in grant applications, unless sex-specific inclusion is unwarranted [25].

U6 snRNA transcription is specifically and significantly elevated in liver tissue of male C57BL/6J mice

The observed gender specific differences in RNA pol III transcription in AC cancer cells (Figure 1) prompted us to investigate whether there are general gender specific differences in RNA pol III transcription *in vivo*. We sought to determine if this was true in normal healthy lung tissue, not cancer, immortalized or transformed cells. Thus, we isolated healthy lung tissue from male and female mice. Endogenous tMet and U6 snRNA transcript levels in both lung and

liver tissue derived from normal 5 week old male and female C57BL/6J mice were analyzed. There was no significant difference in tMet and U6 snRNA transcript expression in normal lung tissue from male and female mice (Figure 2A). This result was surprising as we did note gender specific difference in AC lung cancer cell lines (Figure 1). However, these results highlight the differences in RNA pol III transcription in normal and cancer cells (reviewed in [2]). We selected another normal tissue to determine if the gender specificity observed in lung adenocarcinoma cell lines was also tissue specific by comparing RNA polymerase III transcription levels in male and female lung and liver tissue. We did note a highly statistically significant increase ($p<0.05$) in U6 snRNA transcription in male liver tissue (Figure 2).

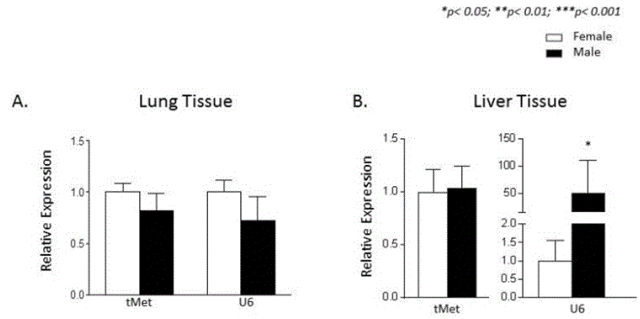

Figure 2: U6 snRNA RNA pol III transcription is elevated in male C57BL/6 liver tissue. Total RNA was isolated from the (A) lung and (B) liver tissues of 5 week female and male C57BL/6J mice. After first strand DNA synthesis, diluted cDNA was used in quantitative real-time PCR using iTaqTM Universal SYBR Green and site specific primers for tMet and U6 snRNA. The ΔΔCt method with GAPDH expression levels was used as a reference for normalization. Meta-analysis of six female and six male C57BL/6 mice was completed using two-tailed students t-test with a 95% confidence interval (Graphpad Prism 3.03); *p<0.05; **p<0.01; ***p<0.001.

There was no significant change in tMet RNA pol III transcription in liver tissue (Figure 2B). Recently, the expression levels of U6 in the carcinoma tissues of the liver and intrahepatic bile ducts were higher than those in the adjacent normal tissues as measured by real time qPCR [26]. Thus, the question arises as to whether an elevated level of U6 snRNA transcription is a key event in the development of liver cancer. The 50-fold increase in U6 snRNA transcription in males may be of significance, as men are on average four times more likely to develop hepatocellular carcinoma (HCC) than women [27]. To the best of our knowledge this is the first report demonstrating gender specific differences in U6 snRNA in normal liver tissue.

p53 expression is significantly decreased in the liver of male C57BL/6J mice

It is well documented that gene external RNA pol III transcription is negatively regulated by a variety of tumor suppressors and oncogenes (reviewed in [2]). The 50-fold U6 snRNA transcript increase observed in male liver tissue prompted us to examine the expression levels of known modulators of RNA pol III transcription to elucidate a possible mechanism (Figure 3). In the lung tissue from normal male and female 5 week old mice, we noted no statistically significant differences in expression of MAF1, RB, p53, BRCA1, CK2, and MAPK (Figure 3A). However, in normal liver tissue isolated from male and female mice, p53 expression is significantly decreased ($p<0.05$) in male mice, but MAF1, RB, BRCA1, CK2, and MAPK levels are not significantly changed (Figure 3B). The significant decrease in p53 expression in male mice can explain, at least in part, the decrease in U6 snRNA transcription (Figure 2B) as TP53 is a known inhibitor of RNA pol III transcription [3-5,28,29] (Figure 3).

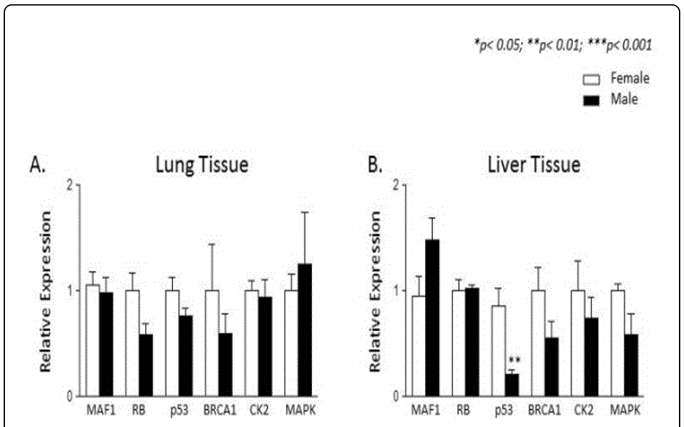

Figure 3: p53 expression is repressed liver tissue from C57BL/6 mice. Total RNA was isolated from the (A) lung and (B) liver tissues of 5 week female and male C57BL/6J mice. After first strand DNA synthesis, diluted cDNA was used in quantitative real-time PCR using iTaqTM Universal SYBR Green and site specific primers for MAF1, RB, p53, BRCA1, CK2 AND MAPK. The ΔΔCt method with GAPDH expression levels was used as a reference for normalization. Meta-analysis of six female and six male C57BL/6 mice was completed using two-tailed students t-test with a 95% confidence interval (Graphpad Prism 3.03); *p<0.05; **p<0.01; ***p<0.001.

The U6 snRNA promoter is specifically demethylated in the liver of male C57BL/6J mice

The gender specific increase in U6 snRNA transcription in C57BL/6J male liver tissue was almost 50-fold (Figure 2B). Thus, we sought to determine if there were additional mechanisms regulating U6 expression, in addition to decreased p53 expression levels (Figure 3B). Previous experiments in breast cancer cell lines have demonstrated that U6 promoter methylation can be regulated by DNMTs [30], suggesting that U6 transcription may be regulated by epigenetic changes. Previously, the methylation status of the U6 promoter has not been determined in normal liver tissue from male C57BL/6J mice. We isolated genomic DNA from C57BL/6J male lung and liver tissue and digested with methylation sensitive restriction enzymes (MSRE). The locations of MSRE cut sites and key regulatory elements in the mouse U6 promoter are denoted in Figure 4A. As a negative control, genomic DNA was digested with BfuAI which has no recognition site in the U6 promoter. Figure 4B shows no significant change in the methylation status on the U6 promoter in male lung tissue, supporting the unchanged U6 expression in male lung tissue (Figure 2A). In male liver tissue, we observed a statistically significant decrease ($p<0.05$) in methylation on the promoter at the Sau3AI site (Figure 4D), located between distal sequence element (DSE) and proximal sequence element (PSE), which may partially account for the significant increase in U6 transcription (Figure 2B). Studies have

demonstrated that well positioned nucleosomes on the U6 promoter enhance transcription by bringing the DSE and PSE in close proximity allowing for cooperative binding of transcription factors to enhance U6 transcription [31,32]. We reason, the decrease in methylation at the Sau3AI site, could further enhance U6 transcription with an endogenous nucleosome. Together, the data presented herein demonstrate that there are gender specific differences in RNA pol III transcription that exist in both lung and liver tissue. These observations warrant further investigation to determine how these differences affect normal regulatory processes between genders and how these differences could potentially drive development of cancer (Figure 4).

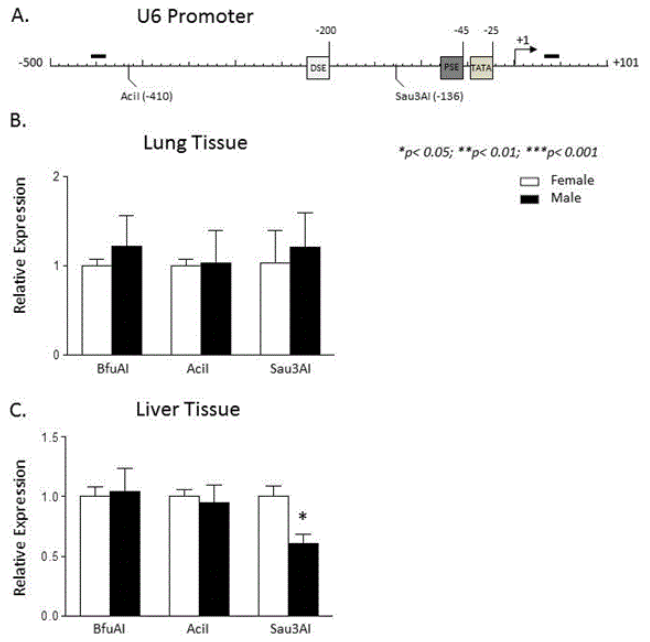

Figure 4: U6 promoter methylation is decreased in liver tissue from C57BL/6J male mice. (A) Mouse U6 promoter schematic with regulatory elements and methylation sensitive restriction enzyme (MSRE) cut sites. MSRE sites on mouse U6 promoter were identified using NEBcutter V2.0. CpG methylation sensitive restriction enzyme cut sites are shown. Transcription start site (+1) is indicated at arrow. Black bars denote the binding sites for primers used in the methylation profile analysis. Genomic DNA was harvested from female and male (B) lung and (C) liver tissue and digested with methylation sensitive restriction enzymes BfuAI, AscI, and Sau3AI. BfuAI was used as a control as no recognition sites have been identified in the mouse U6 promoter. The digestion profile was then analyzed by quantitative real-time PCR using primers spanning the mouse U6 promoter region. The methylation levels were calculated using the ΔΔCt method with GAPDH expression levels used as a reference for normalization. Statistical analysis was performed using two-tailed students t-test with a 95% confidence interval (Graphpad Prism 6); *p<0.05; **p<0.01; ***p<0.001.

Discussion

To the best of our knowledge, this is the first time RNA pol III transcription has been analyzed for gender specific differences. Our finding that EGCG regulates RNA pol III transcription differently in AC cell lines derived from male and female patients is significant (Figure 1). Our observed significant decrease in gene internal and external RNA pol III transcription by EGCG in AC from females (Figure 1A) agrees with our previous work in cervical cancer cells [16].

Nearly 40% of lung cancers in the US are AC, occurring more frequently in nonsmokers and women [33]. Green tea, specifically the polyphenol EGCG has been evaluated as a chemopreventive in a variety of cancers, including lung (reviewed in [34]). However, previous studies have not directly compared EGCG effects on tMet and U6 snRNA transcription in AC lung cancer cell lines derived from male and female patients. Although EGCG inhibits tMet and U6 snRNA transcription in AC lung cancer cells from females (Figure 1A), it significantly stimulates gene internal RNA pol III transcription in AC male derived cancer cells (Figure 1B), suggesting that different mechanisms regulating RNA pol III transcription by EGCG are occurring and require further investigation to determine if dietary sources of EGCG are contraindicated in males with AC lung cancer. Comparison of basal levels of RNA pol III transcription in male and female derived AC cancer cell lines demonstrate that tMet transcription is significantly elevated in the female derived H2347 AC cell line (Figure 1C). The female derived H2347 AC cancer cell line has a 1.7 fold increase in the relative copy number of EGFR as compared to A549 cells [23] which may explain the significant increase in tRNA RNA pol III transcription. The cBioPortal cancer genomics database [35] identified significant amplifications of EGFR in AC lung cancer studies with a majority of EGFR amplifications occurring in female patients. In females with AC lung cancer, 80% (Lung Adenocarcinoma, Broad, Cell 2012), 67% (Lung Adenocarcinoma Study, TCGA, Provisional), and 60% (Lung Adenocarcinoma, TCGA, Nature 2014) had EGRF amplifications [35], suggesting a common mechanism of deregulation of RNA pol III transcription in female derived AC lung cancer (Figure 1A) by EGFR.

Interestingly, the *in vivo* data presented demonstrate that there is not a significant difference in U6 snRNA or tMet expression in lung tissue derived from normal female and male mice (Figure 2). These data imply that RNA pol III transcription in the lung specifically demonstrates gender differences in the context of AC lung cancer and that treatment of AC lung cancer cell lines with the chemopreventive EGCG differentially regulates male and female AC lung cancer (Figure 1). These differences in AC cancer cell lines versus normal tissue warrants further investigation into the gender differences in non-sex specific cancers.

Figure 2 suggests that there are gender specific differences in RNA pol III transcription in liver tissue isolated from mice which, to the best of our knowledge, has never been investigated before. The significant increase in U6 expression (Figure 2B), specific decrease in p53 (Figure 3B) expression and U6 promoter methylation (Figure 4C), in male liver tissue may explain, in part, why males are more susceptible to HCC. U6 transcription is downregulated in HCC [36], suggesting that U6 transcription is decreased in liver injury and may serve as a biomarker for liver injury. The data presented herein argue gender specific differences in RNA pol III transcription exist and warrant further investigation both *in vivo* and *in vitro*.

Author Contributions

ND and JK carried out *in vitro* and *in vivo* experiments. ND, JK, SC, and LS performed data analysis. LS and SC drafted and revised manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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