SNPs in the TGF-β Signaling Pathway Are Associated with Increased Risk of Brain Metastasis in Patients with Non-Small-Cell Lung Cancer

Qianxia Li¹, Huanlei Wu¹, Bei Chen³, Guangyuan Hu¹, Liu Huang¹, Kai Qin¹, Yu Chen¹, Xianglin Yuan¹*, Zhongxing Liao²

¹Department of Oncology, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, Hubei Province, China, ²Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States of America, ³Department of Electrocardiographic Room, Hubei Provincial Tumor Hospital, Wuhan, Hubei Province, China

Abstract

Purpose: Brain metastasis (BM) from non-small cell lung cancer (NSCLC) is relatively common, but identifying which patients will develop brain metastasis has been problematic. We hypothesized that genotype variants in the TGF-β signaling pathway could be a predictive biomarker of brain metastasis.

Patients and Methods: We genotyped 33 SNPs from 13 genes in the TGF-β signaling pathway and evaluated their associations with brain metastasis risk by using DNA from blood samples from 161 patients with NSCLC. Kaplan-Meier analysis was used to assess brain metastasis risk; Cox hazard analyses were used to evaluate the effects of various patient and disease characteristics on the risk of brain metastasis.

Results: The median age of the 116 men and 45 women in the study was 58 years; 62 (39%) had stage IIIb or IV disease. Within 24 months after initial diagnosis of lung cancer, brain metastasis was found in 60 patients (37%). Of these 60 patients, 16 had presented with BM at diagnosis. Multivariate analysis showed the GG genotype of SMAD6: rs12913975 and TT genotype of INHBC: rs4760259 to be associated with a significantly higher risk of brain metastasis at 24 months follow-up (hazard ratio [HR] 2.540, 95% confidence interval [CI] 1.204–5.359, P = 0.014; and HR 1.885, 95% CI 1.086–3.273, P = 0.024), compared with the GA or CT/CC genotypes, respectively. When we analyzed combined subgroups, these rates showed higher for those having both the GG genotype of SMAD6: rs12913975 and the TT genotype of INHBC: rs4760259 (HR 2.353, 95% CI 1.390–3.985, P = 0.001).

Conclusions: We found the GG genotype of SMAD6: rs12913975 and TT genotype of INHBC: rs4760259 to be associated with risk of brain metastasis in patients with NSCLC. This finding, if confirmed, can help to identify patients at high risk of brain metastasis.

Citation: Li Q, Wu H, Chen B, Hu G, Huang L, et al. (2012) SNPs in the TGF-β Signaling Pathway Are Associated with Increased Risk of Brain Metastasis in Patients with Non-Small-Cell Lung Cancer. PLoS ONE 7(12): e51713. doi:10.1371/journal.pone.0051713

Editor: Todd W. Miller, Dartmouth, United States of America

Received August 13, 2012; Accepted November 5, 2012; Published December 17, 2012

Copyright: © 2012 Li et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was funded by the National Natural Science Foundation (grant 81071832), a Key Project of Hubei Provincial Health Office grant (JXSA01), and a Wuhan Planning Project of Science and Technology grant (20116103839-07). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: yxl@medmail.com.cn

Introduction

More 150,000 patients with cancer are diagnosed with brain metastasis each year [1], with the lung being the most common primary site for secondary BM [2,3]. Improved RT techniques and the increased use of combined-modality therapy have reduced distant metastases and significantly improved survival. However, it has shown to be associated with increased rates of overall brain failure [4]. The outcome of the diagnosis of brain metastases is dismal. Even for young patients with good performance status and controlled extra-cranial disease, the median survival time for patients after the development of BM is only about 7 months [5]. Means of preventing the development of BM are therefore urgently sought. For example, prophylactic cranial irradiation (PCI) has a clearly defined role in the treatment of high-risk patients with acute lymphocytic leukemia and patients with small-cell lung cancer (SCLC) [6]. These are radiosensitive tumors where moderate doses of radiation can be employed and results in significant improvements in intracranial control in addition to overall survival, and therefore PCI is considered standard of care. Perhaps patients with non-small cell lung cancer (NSCLC) as well. Prior randomized, controlled trials and several prospective studies without brain primary end points and retrospective studies evaluating PCI for NSCLC have consistently shown a decrease and/or delay in BM with PCI [4,7,8,9]. But PCI has not become part of standard management for LA-NSCLC because of concern for long-term toxicity and lack of a proven survival benefit. It is unclear as to whether this is secondary to failure of identifying the
cohort best suited for prevention. The authors of this paper hypothesized that among NSCLC patients of stage I-IV may exist a group of patients at high risk of presenting BM. This group should be identified in order to serve as target for future studies of PCI application in NSCLC and avoid side effects for those who at low risk of presenting BM.

Defining the cohort of high-risk patients is difficult, because it is dependent on reports that often have conflicting results. Pretreatment factors that predict for high rates of BM include histology, extent of disease, and young age. However, not all studies have shown a significant correlation [10,11,12]. The expression levels of three genes, $CDH2$ (N-cadherin), $KIFCl$, and $FAL2$, was found in one study to be highly predictive of BM in early and advanced lung cancer [13]. The expression levels of genes can be effect by other factors and not so precise which seriously limits this approach for risk prediction. Rarely study addressing the question about the association between polymorphisms and brain failure. Moreover, the heterogeneity and genetic complexity of NSCLC make it unlikely that any single SNP would be sufficient to confer the risk of BM. Rather, studying multiple SNPs in signaling pathways that regulate cell proliferation and migration may be a more powerful way of pinpointing the genes and polymorphisms involved in conferring risk of BM. One such pathway is that of transforming growth factor-β (TGF-β).

The TGF-β superfamily comprises TGF-βs, bone morphoge-netic proteins, activins, and related proteins. TGF-β signaling pathways have diverse effects on cell proliferation, morphogenesis, migration, extracellular matrix production, and apoptosis. In particular, TGF-β suppresses early-stage tumor development by virtue of its potent growth inhibitory effect, but becomes a proto-oncogenic factor that stimulates tumor cell growth and invasive-ness at later stages of tumorigenesis [14,15]. Tumor cells can escape the antiproliferative effects of TGF-β by acquiring mutations in components of signaling pathways or by selectively disrupting TGF-β signaling. The epithelial-to-mesenchymal transition (EMT) is associated with cellular acquisition of motility and invasive properties that promote the formation of distant metastasis [16]. A variety of other mechanisms, including changes in expression of cell-cell adhesion molecules and secretion of metalloproteinases, also contribute to the metastatic process [17].

Given the prominent role of the TGF-β pathway in maintaining cellular function and the effect of its disruption on distant metastasis, common genetic variations in this pathway may emerge as potential predictors of BM risk. In this study, we tested the hypothesis that common genetic variants in the TGF-β pathway are associated with BM risk, and we attempted to identify subgroups of patients with NSCLC who are at particularly high risk of developing BM.

### Patients and Methods

#### Subjects in Population

Subjects in this study were selected from a total of 201 patients with lung cancer who had been treated at either the Tongji Hospital Cancer Center or the Hubei Provincial Tumor Hospital in 2008–2009 who also had blood samples available for analysis. All study participants provided written informed consent before blood samples were collected. The study was approved by the Ethics Committee of Tongji Medical College. Of these 201 patients, 190 had documentation having undergone complete disease staging, and 161 had pathologically confirmed NSCLC. These 161 patients were the basis of this analysis. Clinical data were obtained from patients’ files. Disease had been staged in terms of the tumor/node/metastasis system in the sixth (2002) edition of the American Joint Committee on Cancer staging manual. The diagnosis of BM was based on computed tomography or magnetic resonance imaging records. The smoking status includes current, former, or never smoker. Former smokers were defined as individuals who had successfully ceased smoking for at least 1 year at the time of study registration. Never smokers were defined as individuals who had <20 total cigarettes during their lifetime [18]. The time to BM was the interval from the date of NSCLC diagnosis to the date of BM diagnosis. The follow-up time was the interval from NSCLC diagnosis to death or to the last hospital visit.

#### Genotyping Methods

The procedures used to select SNPs in the TGF-β pathway have been described previously [19]. Briefly, we used databases at Gene

---

**Table 1. Genes and single-nucleotide polymorphisms (SNPs) selected for analysis.**

| Gene (number of SNPs) | SNP       | Allelic change |
|-----------------------|-----------|----------------|
| TGFBR1 (3)            | rs 4803455 | A>C            |
|                       | rs 1800469 | C>T            |
|                       | rs 1800470 | C>T            |
| BMP1 (2)              | rs 3857979 | C>T            |
| BMP2 (1)              | rs 7838961 | A>G            |
| BMP4 (2)              | rs 235756  | C>T            |
|                      | rs 17563   | C>T            |
| INHBC (1)             | rs 8014071 | G>T            |
|                      | rs 4760259 | C>T            |
|                      | rs 10819638| C>T            |
|                      | rs 6478974 | A>T            |
|                      | rs 10733170| A>G            |
| SMAD1 (1)             | rs 11939979| A>C            |
| SMAD3 (7)             | rs 4776342 | A>G            |
|                      | rs 12102171| A>C            |
|                      | rs 6494633 | C>T            |
|                      | rs 11632964| C>T            |
|                      | rs 750766  | A>G            |
|                      | rs 4776343 | A>G            |
|                      | rs 11071938| C>T            |
| SMAD4 (6)             | rs 948588  | A>G            |
|                      | rs 1231457 | A>G            |
|                      | rs 7244227 | A>G            |
|                      | rs 12455792| C>T            |
|                      | rs 12858604| A>G            |
|                      | rs 10129713| A>G            |
| SMAD6 (3)             | rs 12913975| A>G            |
|                      | rs 12906898| A>G            |
|                      | rs 4776318 | A>C            |
| SMAD7 (1)             | rs 7227023 | A>G            |
| SMAD8 (2)             | rs 7333607 | A>G            |
|                      | rs 511674  | A>G            |

NOTE. A total of 33 SNPs from 13 TGF-β pathway-related genes were genotyped.

doi:10.1371/journal.pone.0105713.t001

---
Oncology (http://www.geneontology.org) and the National Center for Biotechnology Information (NCBI)'s Gene database (http://www.ncbi.nlm.nih.gov/gene) and related literature to identify all functional single nucleotide polymorphisms (SNPs) of the genes in TGF-β signaling pathways with a minor allele frequency of more than 0.05 in a Chinese population. We selected 33 SNPs in 13 genes related to TGF-β pathways that were either located in the promoter untranslated region or coding region of the gene or had been previously reported as being associated with survival, lung cancer, or general metastasis (Table 1). Genomic DNA was isolated from peripheral blood lymphocytes by using the QuickGene DNA whole blood kit S (Fuji Film) and stored at –80°C until use. Thirty-two of the SNPs were genotyped by using MALDI-TOF mass spectrophotometry to detect allele-specific primer extension products with the MassARRAY platform (Sequenom, Inc.). Assay data were analyzed using Sequenom TYPER software (version 4.0). The individual call rate threshold was at least 95%. The 33rd SNP (TGFB1: rs1800470) was genotyped by using the TaqMan assay [20].

Statistical Analysis

This analysis was undertaken after all patients had been potentially observed for a minimum of 24 months. Patients were grouped according to genotype. Statistical analysis was performed using SPSS (version 16.0) software. Cox proportional hazards model was used to calculate hazard ratio (HR) and 95% confidence intervals (CIs) for multivariate survival analyses, while adjusting for sex, age, disease stage, tumor histology, Karnofsky performance status (KPS), and smoking status. Kaplan-Meier plots and log rank tests were used to estimate the effect of genotype on BM risk. Likelihood ratio tests were used for each multivariate Cox regression to assess goodness-of-fit. A P value of ≤0.05 was considered to indicate statistical significance in two-sided t tests.

Results

Patient Characteristics

Characteristics of the 161 patients (116 men and 45 women) are shown in Table 2. The median age was 58 years (range, 32 to 80 years); 61% had stage IIIA disease; 60% had adenocarcinoma, and 54% had smoked tobacco (71.6% in male and 8.9% in female).

Brain Metastasis and Genotypes

The median time from NSCLC diagnosis to detection of BM was 7.5 months (range, 0 to 23 months). The median time was 10 months when patients who presented with BM were excluded. Associations between patient- and tumor-related characteristics and BM by univariate and multivariate analyses are shown in Table 2. As expected, disease stage was associated with BM, with patients having stage IIIB or stage IV disease at higher risk of BM (P<0.010). And patients with adenocarcinoma were associated with higher risk of BM by Cox hazard analyses (P=0.032). However, the smoking status has no association with BM risk in this population.

| Characteristic | No. of Patients (%) | HR | Univariate Analysis (95% CI) | P Value | HR | Multivariate Analysis (95% CI) | P Value |
|----------------|---------------------|----|-------------------------------|---------|----|--------------------------------|---------|
| Sex            |                     |    |                               |         |    |                                |         |
| Female         | 45 (28)             | 1.00| 0.83 (0.48–1.42)               | 0.493   | 0.83| 0.45 (0.27–1.53)               | 0.572   |
| Male           | 116 (72)            | 0.827| 0.73 (0.48–1.14)                | 0.980   | 0.457–1.734                  | 0.732   |
| Age, years     |                     |    |                               |         |    |                                |         |
| ≥60 years      |                     |    |                               |         |    |                                |         |
| <60 years      |                     |    |                               |         |    |                                |         |
| Median (range) | 58 (32–80)          | 1.00| 0.73 (0.48–1.14)                | 0.457   | 0.457–1.734                  | 0.732   |
| Disease stage at diagnosis | |    |                               |         |    |                                |         |
| I, II, IIIA    | 99 (61)             | 1.00| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| IIIB, IV       | 62 (39)             | 3.796| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| Tumor histology|                     |    |                               |         |    |                                |         |
| Squamous cell  | 51 (32)             | 1.00| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| Adenocarcinoma | 97 (60)             | 1.968| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| NSCLC, NOS     | 13 (8)              | 0.895| 0.86 (0.52–1.40)                | 0.315   | 0.425–2.577                  | 0.921   |
| KPS            |                     |    |                               |         |    |                                |         |
| >80            | 22 (14)             | 1.00| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| 80             | 87 (54)             | 1.560| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| <80            | 52 (32)             | 1.538| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| Smoking status/Tobacco use | |    |                               |         |    |                                |         |
| current        | 62 (38)             | 1.00| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| former         | 25 (16)             | 1.965| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |
| never          | 74 (46)             | 1.261| 1.06 (0.65–1.71)                | 0.315   | 0.425–2.577                  | 0.921   |

NOTE. Multivariate analyses were adjusted for all factors listed in Table. Abbreviations: HR, hazard ratio; CI, confidence interval; NSCLC NOS, non-small cell lung cancer, not otherwise specified; KPS, Karnofsky performance status.

doi:10.1371/journal.pone.0051713.t002
Figure 1 illustrate cumulative BM-free survival rates for all patients according to genotype. These rates remained lower for those with either the GG genotype of \textit{SMAD6}: rs12913975 ($P=0.024$, Figure 1A) or the TT genotype of \textit{INHBC}: rs4760259 ($P=0.045$, Figure 1B). When we analyze combined subgroups, these rates showed lower for those having both the GG genotype of \textit{SMAD6}: rs12913975 and the TT genotype of \textit{INHBC}: rs4760259 ($P=0.003$, Figure 1C). Other 31 SNPs in the TGF-$\beta$ pathway in Table 1 were also analyzed, but no significant correlation was found ($P=0.877$, Figure 1D for \textit{TGFB1}: rs4803455; date of other 30 selected SNPs not shown).

Table 3 and 4 lists the findings of Kaplan-Meier analyses of BM incidence according to genotype at 24 months from diagnosis. In general, BM developed more often in patients with the GG genotype of \textit{SMAD6}: rs12913975 (43%) or the TT genotype of \textit{INHBC}: rs4760259 (44%) compared with the GA (21%) or CT/CC genotypes (27%). These associations between genotype and BM were statistically significant for both subgroups. When we analyze combined subgroups, we can see having both GG

### Table 3. Associations between genotypes and brain metastases.

| Polymorphisms and Genotypes | No. of Patients (All) | No. of Events (%) | HR  | 95% CI          | $P$ Value | No. of Patients without BM at Diagnosis | No. of Events (%) | HR | 95% CI | $P$ Value |
|-----------------------------|----------------------|------------------|-----|-----------------|-----------|----------------------------------------|------------------|----|--------|-----------|
| \textit{SMAD6}: rs12913975  |                      |                  |     |                 |           |                                        |                  |     |        |           |
| GA                          | 38                   | 8 (21)           | 1.00|                 |           | 36                                     | 6 (17)           | 1.00|        |           |
| GG                          | 122                  | 52 (43)          | 2.54| 1.204–5.359     | 0.014     | 108                                    | 38 (35)          | 2.577| 1.086–6.117 | 0.032     |
| \textit{INHBC}: rs4760259   |                      |                  |     |                 |           |                                        |                  |     |        |           |
| CT or CC                    | 69                   | 19 (27)          | 1.00|                 |           | 64                                     | 14 (22)          | 1.00|        |           |
| TT                          | 91                   | 40 (44)          | 1.885| 1.086–3.273     | 0.024     | 81                                     | 30 (37)          | 1.961| 1.032–3.727 | 0.040     |

NOTE. Multivariate analyses in this table were adjusted for Stage, Histology, Age, and Smoking status. Similar results were obtained when multivariate analyses were adjusted for all the factors listed in Table1 (data not shown).

Abbreviations: HR, hazard ratio; CI, confidence interval; BM, brain metastases.

doi:10.1371/journal.pone.0051713.t003
polymorphism in ovarian cancer [23], breast cancer and pancreatic carcinoma; mechanisms. Variants in both proteins may be involved in metastasis via similar mechanism in the TGF-

The SMAD6 and SMAD7 proteins may contribute to metastases, include CNS metastases. We also found INHBC: rs4760259 polymorphisms to be associated with BM risk. The INHBC gene is located on human chromosome 12, region q13.1, and encodes a protein named βC, belonging to the inhibin subgroup. Inhibitin and activin proteins, along with various growth and differentiation factors, Muellerian inhibiting substance, and other proteins, belong to the TGF-β superfamily. Activins have many effects on mesoderm formation [25], cell proliferation and apoptosis [26], branching morphogenesis [27], inflammation [28] and reproduction [29]. One α-subunit and four β-subunit isoforms (βA, BB, BC and BE) have been found in mammals and humans [30]. The activin α, βA, and BB subunits, and their homo- and heterodimers have been well characterized; activin A (βA/ βA), for example, is a pleiotropic protein that affects apoptosis, cell-cycle control, angiogenesis and immune suppression [31]. The precise role of the βC subunit, however, is unclear. Activin βC subunit mRNA has been detected in rat and mouse lung, epididymis, testis, uterus, spleen, posterior pituitary, and adrenal gland, and in human ovary, testis, placenta, and prostate [31]. The activin βC subunit or its dimers may oppose the action of activin A. In one in vitro study, the activin βC subunit had a pro-apoptotic effect in liver cell lines. Furthermore, the activin βC subunit was downregulated in prostate and liver tumor cell lines [32]. Transfection of the activin βC subunit into the PC3 prostate cancer cell line resulted in decreased activin A levels [33]. A recent study showed polymorphisms in INHBC is associated with ovarian cancer risk [34]. Another study showed it to be strongly associated with survival in NSCLC [24]. It can be seen that activin βC subunit is associate with tumorigenesis and progress, and metastases is an important step in tumor progression which strongly associated with poor prognosis, therefore we can believe SNPs in INHBC may contribute to BM.

A single SNP often provides a modest or undetectable effect whereas the amplified effects of combined SNPs in the same pathway may enhance predictive power. We analyzed the association with BM in patients with both two genotypes. A clear and significant trend was evident for higher risk with the combined associations between any other genotype and the incidence of BM (Table 3 and 4).

Table 3 and 4).

Similar analyses of the other 31 selected SNPs showed no associations between any other genotype and the incidence of BM (Table 3 and 4).

Discussion

In this study, we systematically evaluated associations between a comprehensive panel of genetic variants in TGF-β signaling pathway genes and BM risk. We found that SNPs in SMAD6: rs12913975 GG or INHBC: rs4760259 TT were associated with the incidence of BM. To the best of our knowledge, this is the first evidence showing this association in patients with lung cancer. With validation, this test could be used as a predictive biomarker to identify patients at high risk of developing brain metastasis during the first 24 months after diagnosis.

One of the polymorphisms we found to be associated with BM risk was in SMAD6, which encodes a protein that localizes to both nuclei and cytoplasm. Sma6 and Smad7 act as “inhibitory” Smads, inhibiting TGF-β family signaling [21]. Induction of Smad6 and Smad7 expression by bone morphogenetic protein and TGF-β signaling represents an auto-inhibitory feedback mechanism in the TGF-β pathway [21]. SMAD6 is expressed in most human tissues, including the lung, but its function in tumorigenesis is not yet established. A previous retrospective study showed that SMAD7 overexpression is linked with a reduced incidence of bone metastases from melanoma and breast cancer [22]. The structural similarity between SMAD6 and SMAD7 proteins suggests that both proteins may be involved in metastasis via similar mechanisms. Variants in SMAD6 have been linked with prognosis in ovarian cancer [23], breast cancer and pancreatic carcinoma; polymorphism in SMAD6 have also been linked with survival in NSCLC [24]. Metastases, especially brain metastases, is an important factor associated with poor prognosis, and SNPs in SMAD6 may contribute to metastases, include CNS metastases.

We also found INHBC: rs4760259 polymorphisms to be associated with BM risk. The INHBC gene is located on human chromosome 12, region q13.1, and encodes a protein named βC, belonging to the inhibin subgroup. Inhibitin and activin proteins, along with various growth and differentiation factors, Muellerian inhibiting substance, and other proteins, belong to the TGF-β superfamily. Activins have many effects on mesoderm formation [25], cell proliferation and apoptosis [26], branching morphogenesis [27], inflammation [28] and reproduction [29]. One α-subunit and four β-subunit isoforms (βA, BB, BC and BE) have been found in mammals and humans [30]. The activin α, βA, and BB subunits, and their homo- and heterodimers have been well characterized; activin A (βA/βA), for example, is a pleiotropic protein that affects apoptosis, cell-cycle control, angiogenesis and immune suppression [31]. The precise role of the βC subunit, however, is unclear. Activin βC subunit mRNA has been detected in rat and mouse lung, epididymis, testis, uterus, spleen, posterior pituitary, and adrenal gland, and in human ovary, testis, placenta, and prostate [31]. The activin βC subunit or its dimers may oppose the action of activin A. In one in vitro study, the activin βC subunit had a pro-apoptotic effect in liver cell lines. Furthermore, the activin βC subunit was downregulated in prostate and liver tumor cell lines [32]. Transfection of the activin βC subunit into the PC3 prostate cancer cell line resulted in decreased activin A levels [33]. A recent study showed polymorphisms in INHBC is associated with ovarian cancer risk [34]. Another study showed it to be strongly associated with survival in NSCLC [24]. It can be seen that activin βC subunit is associate with tumorigenesis and progress, and metastases is an important step in tumor progression which strongly associated with poor prognosis, therefore we can believe SNPs in INHBC may contribute to BM.

A single SNP often provides a modest or undetectable effect whereas the amplified effects of combined SNPs in the same pathway may enhance predictive power. We analyzed the association with BM in patients with both two genotypes. A clear and significant trend was evident for higher risk with the combined subgroups. These results suggest a cumulative influence by multiple genetic variants within the TGF-β signaling pathway were able to further enhance predictive power.

However, neither of the SNPs we identified as being linked with BM is located in the coding region, which suggest that these SNPs may not affect SMAD6 or INHBC function directly but rather may change levels of gene expression through being located in regulatory regions or through linkage to other SNPs that affect gene activity. Further in vitro and in vivo functional studies are needed to confirm the functional significance of the identified SMAD6 and INHBC SNPs.

Moreover, we found that 48% of patients with both high risk alleles do not have brain metastasis (Table 4), and 1.7% (1/60) of patients with brain metastasis do not carry either of the two high risk genotypes. As the heterogeneity and genetic complexity of
Table 5. Associations between genotypes and brain metastases (the other 31 selected SNPs).

| Polymorphisms and Genotypes | No. of Patients (All) | No. of Events (%) | HR   | 95% CI          | P value |
|-----------------------------|-----------------------|-------------------|------|-----------------|---------|
| TGFB1: rs4803455            |                       |                   |      |                 |         |
| CA or AA                    | 96                    | 37(39)            | 1.00 |                 |         |
| CC                          | 62                    | 23(37)            | 1.13 | 0.670–1.929     | 0.634   |
| TGFB1: rs1800469            |                       |                   |      |                 |         |
| CT or CC                    | 118                   | 45(38)            | 1.00 |                 |         |
| TT                          | 41                    | 15(37)            | 1.22 | 0.674–2.216     | 0.510   |
| TGFB1: rs1800470            |                       |                   |      |                 |         |
| CT or TT                    | 112                   | 42(38)            | 1.00 |                 |         |
| CC                          | 45                    | 17(38)            | 1.18 | 0.662–2.103     | 0.575   |
| BMP1: rs3857979             |                       |                   |      |                 |         |
| CT or TT                    | 72                    | 23(32)            | 1.00 |                 |         |
| CC                          | 89                    | 37(42)            | 1.29 | 0.768–2.196     | 0.330   |
| BMP1: rs7838961             |                       |                   |      |                 |         |
| GA or GG                    | 75                    | 26(35)            | 1.00 |                 |         |
| AA                          | 86                    | 34(40)            | 1.16 | 0.693–1.959     | 0.564   |
| BMP2: rs235756              |                       |                   |      |                 |         |
| TC or CC                    | 48                    | 19(40)            | 1.00 |                 |         |
| TT                          | 113                   | 41(37)            | 0.93 | 0.538–1.613     | 0.800   |
| BMP4: rs17563               |                       |                   |      |                 |         |
| TC or CC                    | 88                    | 31(35)            | 1.00 |                 |         |
| TT                          | 73                    | 29(40)            | 0.92 | 0.541–1.565     | 0.759   |
| BMP4: rs8014071             |                       |                   |      |                 |         |
| AG or GG                    | 93                    | 38(41)            | 1.00 |                 |         |
| AA                          | 65                    | 22(34)            | 0.89 | 0.524–1.513     | 0.667   |
| TGFB1: rs10819638           |                       |                   |      |                 |         |
| CT or TT                    | 102                   | 37(36)            | 1.00 |                 |         |
| CC                          | 59                    | 23(39)            | 1.39 | 0.812–2.393     | 0.229   |
| TGFB1: rs6478974            |                       |                   |      |                 |         |
| TA or AA                    | 86                    | 29(34)            | 1.00 |                 |         |
| TT                          | 75                    | 31(41)            | 1.00 | 0.602–1.682     | 0.981   |
| TGFB1: rs10733710           |                       |                   |      |                 |         |
| GA or AA                    | 69                    | 30(44)            | 1.00 |                 |         |
| GG                          | 91                    | 29(32)            | 0.69 | 0.407–1.171     | 0.169   |
| ACVR2A: rs1424954           |                       |                   |      |                 |         |
| GA or GG                    | 111                   | 42(38)            | 1.00 |                 |         |
| AA                          | 49                    | 18(37)            | 1.00 | 0.572–1.762     | 0.988   |
| SMAD1: rs11939979           |                       |                   |      |                 |         |
| CA or CC                    | 63                    | 24(38)            | 1.00 |                 |         |
| AA                          | 95                    | 35(37)            | 0.94 | 0.552–1.624     | 0.843   |
| SMAD3:rs4776342             |                       |                   |      |                 |         |
| AG or AA                    | 112                   | 42(38)            | 1.00 |                 |         |
| GG                          | 49                    | 18(37)            | 1.00 | 0.607–1.852     | 0.836   |
| SMAD3:rs12102171            |                       |                   |      |                 |         |
| CT or TT                    | 90                    | 34(38)            | 1.00 |                 |         |
| CC                          | 71                    | 26(37)            | 1.16 | 0.685–1.952     | 0.587   |
| SMAD3:rs6494633             |                       |                   |      |                 |         |
| CT                          | 33                    | 11(33)            | 1.00 |                 |         |
| CC                          | 128                   | 49(38)            | 0.80 | 0.404–1.588     | 0.526   |
| Polymorphisms and Genotypes | No. of Patients (All) | No. of Events (%) | HR    | 95% CI      | \( P \) value   |
|-----------------------------|----------------------|-------------------|-------|-------------|-----------------|
| TC or TT                    | 94                   | 37(39)            | 1.000 |             |                 |
| CC                          | 67                   | 23(34)            | 0.755 | 0.445–1.282 | 0.298           |
| SMAD3 rs750766              |                      |                   |       |             |                 |
| AG or AA                    | 98                   | 35(36)            | 1.000 |             |                 |
| GG                          | 62                   | 25(40)            | 1.215 | 0.721–2.048 | 0.465           |
| SMAD3 rs4776343             |                      |                   |       |             |                 |
| AG                          | 14                   | 6(43)             | 1.000 |             |                 |
| GG                          | 147                  | 54(37)            | 0.931 | 0.395–2.192 | 0.869           |
| SMAD3 rs11071938            |                      |                   |       |             |                 |
| TC or TT                    | 72                   | 27(38)            | 1.000 |             |                 |
| CC                          | 89                   | 33(37)            | 1.077 | 0.646–1.795 | 0.776           |
| SMAD4 rs948588              |                      |                   |       |             |                 |
| GA or AA                    | 17                   | 9(53)             | 1.000 |             |                 |
| GG                          | 144                  | 51(35)            | 0.740 | 0.359–1.523 | 0.413           |
| SMAD4 rs12456284            |                      |                   |       |             |                 |
| AG or GG                    | 93                   | 34(37)            | 1.000 |             |                 |
| AA                          | 65                   | 24(37)            | 0.845 | 0.494–1.444 | 0.537           |
| SMAD4 rs7244227             |                      |                   |       |             |                 |
| AG or GG                    | 109                  | 41(38)            | 1.000 |             |                 |
| AA                          | 50                   | 19(38)            | 0.905 | 0.521–1.571 | 0.722           |
| SMAD4 rs12455792            |                      |                   |       |             |                 |
| CT or TT                    | 110                  | 45(41)            | 1.000 |             |                 |
| CC                          | 49                   | 15(31)            | 0.612 | 0.338–1.107 | 0.104           |
| SMAD4 rs12958604            |                      |                   |       |             |                 |
| AG or GG                    | 125                  | 47(38)            | 1.000 |             |                 |
| AA                          | 36                   | 13(36)            | 0.828 | 0.446–1.540 | 0.552           |
| SMAD4 rs10502913            |                      |                   |       |             |                 |
| AG or AA                    | 87                   | 32(37)            | 1.000 |             |                 |
| GG                          | 74                   | 28(38)            | 0.920 | 0.549–1.541 | 0.920           |
| SMAD6: rs12906898           |                      |                   |       |             |                 |
| AG or AA                    | 37                   | 13(35)            | 1.000 |             |                 |
| GG                          | 122                  | 47(39)            | 1.297 | 0.688–2.444 | 0.421           |
| SMAD6: rs4776318            |                      |                   |       |             |                 |
| CA or AA                    | 57                   | 20(35)            | 1.000 |             |                 |
| CC                          | 103                  | 40(39)            | 1.161 | 0.662–2.037 | 0.603           |
| SMAD7: rs7227023            |                      |                   |       |             |                 |
| GA                          | 8                    | 4(50)             | 1.000 |             |                 |
| GG                          | 153                  | 56(37)            | 0.808 | 0.284–2.301 | 0.690           |
| SMAD8: rs7333607            |                      |                   |       |             |                 |
| AG                          | 29                   | 12(41)            | 1.000 |             |                 |
| AA                          | 128                  | 48(38)            | 0.942 | 0.497–1.786 | 0.855           |
| SMAD8: rs511674             |                      |                   |       |             |                 |
| GA                          | 30                   | 10(33)            | 1.000 |             |                 |
| AA                          | 131                  | 50(38)            | 1.341 | 0.678–2.653 | 0.399           |

NOTE. Multivariate analyses in this table were adjusted for Stage, Histology, Age, and Smoking status. Similar results were obtained when multivariate analyses were adjusted for all the factors listed in Table 1 (data not shown). Abbreviations: HR, hazard ratio; CI, confidence interval.

doi:10.1371/journal.pone.0051713.t005
Table 6. Associations between genotypes and brain metastases, excluding those with brain metastases at initial diagnosis of non-small cell lung cancer (the other 31 selected SNPs).

| Polymorphisms and Genotypes | No. of Patients (All) | No. of Events (%) | HR   | 95% CI      | P value |
|-----------------------------|-----------------------|------------------|------|-------------|---------|
| **TGFB1**: rs4803455        |                       |                  |      |             |         |
| CA or AA                    | 85                    | 26(31)           | 1.000|             |         |
| CC                          | 57                    | 18(32)           | 1.257| 0.680–2.326 | 0.466   |
| **TGFB1**: rs1800469        |                       |                  |      |             |         |
| CT or CC                    | 105                   | 32(31)           | 1.000|             |         |
| TT                          | 38                    | 12(32)           | 1.335| 0.679–2.628 | 0.402   |
| **TGFB1**: rs1800470        |                       |                  |      |             |         |
| CT or TT                    | 100                   | 30(30)           | 1.000|             |         |
| CC                          | 41                    | 13(32)           | 1.187| 0.608–2.320 | 0.615   |
| **BMP1**: rs3857979         |                       |                  |      |             |         |
| CT or TT                    | 67                    | 18(27)           | 1.000|             |         |
| CC                          | 78                    | 26(33)           | 1.249| 0.682–2.289 | 0.472   |
| **BMP1**: rs7838961         |                       |                  |      |             |         |
| GA or GG                    | 69                    | 20(29)           | 1.000|             |         |
| AA                          | 76                    | 24(32)           | 1.138| 0.622–2.083 | 0.676   |
| **BMP2**: rs235756          |                       |                  |      |             |         |
| TC or CC                    | 41                    | 12(29)           | 1.000|             |         |
| TT                          | 104                   | 32(31)           | 1.108| 0.569–2.158 | 0.762   |
| **BMP4**: rs17563           |                       |                  |      |             |         |
| TC or CC                    | 77                    | 20(26)           | 1.000|             |         |
| TT                          | 68                    | 24(33)           | 1.312| 0.707–2.435 | 0.389   |
| **BMP4**: rs8014071         |                       |                  |      |             |         |
| AG or GG                    | 81                    | 26(32)           | 1.000|             |         |
| AA                          | 61                    | 18(30)           | 0.995| 0.543–1.823 | 0.986   |
| **TGFBR1**: rs10819638      |                       |                  |      |             |         |
| CT or TT                    | 91                    | 26(29)           | 1.000|             |         |
| CC                          | 54                    | 18(33)           | 1.555| 0.832–2.907 | 0.166   |
| **TGFBR1**: rs6478974       |                       |                  |      |             |         |
| TA or AA                    | 76                    | 19(25)           | 1.000|             |         |
| TT                          | 69                    | 25(36)           | 1.245| 0.679–2.285 | 0.479   |
| **TGFBR1**: rs10733710      |                       |                  |      |             |         |
| GA or AA                    | 62                    | 23(37)           | 1.000|             |         |
| GG                          | 83                    | 21(25)           | 0.644| 0.350–1.185 | 0.157   |
| **ACVR2A**: rs1424954       |                       |                  |      |             |         |
| GA or GG                    | 102                   | 33(32)           | 1.000|             |         |
| AA                          | 42                    | 11(26)           | 0.757| 0.376–1.523 | 0.436   |
| **SMAD1**: rs11939979       |                       |                  |      |             |         |
| CA or CC                    | 56                    | 17(30)           | 1.000|             |         |
| AA                          | 86                    | 26(30)           | 0.989| 0.526–1.860 | 0.974   |
| **SMAD3**: rs4776342        |                       |                  |      |             |         |
| AG or AA                    | 102                   | 32(31)           | 1.000|             |         |
| GG                          | 43                    | 12(28)           | 0.928| 0.475–1.811 | 0.826   |
| **SMAD3**: rs12102171       |                       |                  |      |             |         |
| CT or TT                    | 79                    | 23(29)           | 1.000|             |         |
| CC                          | 66                    | 21(32)           | 1.303| 0.711–2.386 | 0.392   |
| **SMAD3**: rs6494633        |                       |                  |      |             |         |
| CT                          | 31                    | 9(29)            | 1.000|             |         |
| CC                          | 114                   | 35(31)           | 0.737| 0.340–1.594 | 0.437   |
| Polymorphisms and Genotypes | No. of Patients (All) | No. of Events (%) | HR  | 95% CI           | P value |
|-----------------------------|----------------------|-------------------|-----|------------------|---------|
| SMAD3: rs11632964           |                      |                   |     |                  |         |
| TC or TT                    | 84                   | 27(32)            | 1.000 |                  |         |
| CC                          | 61                   | 17(28)            | 0.736 | 1.395–1.372      | 0.335   |
| SMAD3: rs750766              |                      |                   |     |                  |         |
| AG or AA                    | 89                   | 26(29)            | 1.000 |                  |         |
| GG                          | 55                   | 18(33)            | 1.218 | 0.661–2.243      | 0.527   |
| SMAD3: rs4776343            |                      |                   |     |                  |         |
| AG                          | 13                   | 5(38)             | 1.000 |                  |         |
| GG                          | 132                  | 39(30)            | 0.780 | 0.304–2.001      | 0.605   |
| SMAD3: rs11071938           |                      |                   |     |                  |         |
| TC or TT                    | 63                   | 18(29)            | 1.000 |                  |         |
| CC                          | 82                   | 26(32)            | 1.274 | 0.696–2.331      | 0.432   |
| SMAD4: rs948588             |                      |                   |     |                  |         |
| GA or AA                    | 14                   | 5(43)             | 1.000 |                  |         |
| GG                          | 131                  | 38(29)            | 0.725 | 0.300–1.753      | 0.476   |
| SMAD4: rs12456284           |                      |                   |     |                  |         |
| AG or GG                    | 84                   | 25(30)            | 1.000 |                  |         |
| AA                          | 59                   | 18(31)            | 0.884 | 0.473–1.651      | 0.699   |
| SMAD4: rs7244227            |                      |                   |     |                  |         |
| AG or GG                    | 99                   | 31(33)            | 1.000 |                  |         |
| AA                          | 44                   | 13(30)            | 0.821 | 0.424–1.587      | 0.821   |
| SMAD4: rs12455792           |                      |                   |     |                  |         |
| CT or TT                    | 98                   | 33(34)            | 1.000 |                  |         |
| CC                          | 45                   | 11(24)            | 0.596 | 0.298–1.192      | 0.143   |
| SMAD4: rs12958604           |                      |                   |     |                  |         |
| AG or GG                    | 112                  | 34(30)            | 1.000 |                  |         |
| AA                          | 33                   | 10(30)            | 0.897 | 0.438–1.837      | 0.767   |
| SMAD4: rs10502913           |                      |                   |     |                  |         |
| AG or AA                    | 79                   | 24(30)            | 1.000 |                  |         |
| GG                          | 66                   | 20(30)            | 0.893 | 0.487–1.638      | 0.714   |
| SMAD6: rs12906898           |                      |                   |     |                  |         |
| AG or AA                    | 34                   | 10(29)            | 1.000 |                  |         |
| GG                          | 109                  | 34(31)            | 1.226 | 0.592–2.539      | 0.583   |
| SMAD6: rs4776318            |                      |                   |     |                  |         |
| CA or AA                    | 52                   | 15(29)            | 1.000 |                  |         |
| CC                          | 92                   | 29(32)            | 1.161 | 0.609–2.216      | 0.650   |
| SMAD7: rs7227023            |                      |                   |     |                  |         |
| GA                          | 7                    | 3(43)             | 1.000 |                  |         |
| GG                          | 138                  | 41(30)            | 0.774 | 0.232–2.590      | 0.678   |
| SMAD8: rs7333607            |                      |                   |     |                  |         |
| AG                          | 25                   | 8(32)             | 1.000 |                  |         |
| AA                          | 116                  | 36(31)            | 1.043 | 0.480–2.265      | 0.916   |
| SMAD8: rs511674             |                      |                   |     |                  |         |
| GA                          | 28                   | 8(29)             | 1.000 |                  |         |
| AA                          | 117                  | 36(31)            | 1.282 | 0.592–2.777      | 0.529   |

NOTE. Multivariate analyses in this table were adjusted for Stage, Histology, Age, and Smoking status. Similar results were obtained when multivariate analyses were adjusted for all the factors listed in Table 1 (data not shown). Abbreviations: HR, hazard ratio; CI, confidence interval.
doi:10.1371/journal.pone.0051713.t006
NSCLC, we speculate that a few other factors and SNPs in other signaling pathways that regulate cell proliferation and migration may be also associated with the risk of BM. Future research are needed to further enhance predictive power. PCl is considered part of standard therapy for limited-stage SCLC, as up to 50% of these patients develop BM [35,36]. Slotman et al. conducted a trial of 286 patients with extensive-stage SCLC who were randomized to receive either PCl or no PCl. Survival at 1 year improved from 13.3 to 27.1% [6]. At this time, nearly all patients with SCLC should be offered PCl to reduce the chance of BM and improve overall survival. Currently, the intensity of prophylactic therapy for acute lymphoblastic leukemia is adjusted to the risk of central nervous system (CNS) relapse [37]. Prior randomized, controlled trials [7,8,9] and several prospective studies evaluating PCl for NSCLC have been published. Studies have significantly show that PCl administered to patients improves intracranial disease control. However, none of these studies have ever shown a survival benefit with the application of PCl. The most recent RTOG trial (0214) evaluating the role of PCl in LA-NSCLC unfortunately closed early due to poor accrual. While there was a promising outcome in decreased intracranial metastases in the treatment group, this failed to result in a survival benefit [4]. Joseph A et al. analyzes it is unclear as to whether this is secondary to failure of identifying the cohort best suited for prevention, the inability of radiation to effect sufficient intracranial disease prevention because of a relatively radio-resistant tumor, or the need for more effective systemic therapies to control extracranial disease so that patients’ survival is long enough to see the benefit of PCl [1]. Because there is no predictive test to identify patients with high risk of brain metastatic, PCl has been given unselectively to all patients. PCI could negatively affect neurocognitive function and quality of life in those patients who do not need PCI [4]. If the findings from current study are validated in a study with adequate statistical power prospectively, we could use the SNPs identified in this study as a pretreatment test to select patients who would benefit from PCl, while avoiding PCl in patients who do not need it.

Our study had some limitations. The small number of patients raises the possibility that some of our findings were due to chance. Future studies are necessary to identify functional significance of the genetic variants we have identified, as well as to confirm or externally evaluate the associations in independent populations.

**Conclusions**

We found that the GG genotype of **SMAD6**: rs12913975 and the TT genotype of **INHBC**: rs4760259 were associated with the incidence of BM in patients with NSCLC. These findings were confirmed in both Kaplan-Meier and multivariate Cox proportional hazard analyses, the latter adjusted for disease stage, tumor histology, age, and smoking status of the patient. These findings may be useful in future efforts to identify patients at high risk of brain metastasis.

**Author Contributions**

Conceived and designed the experiments: QL, XY. Performed the experiments: QL, XY, ZL. Contributed reagents/materials/analysis tools: HW, BC, YC, GH, LH, KQ. Wrote the paper: QL, XY, ZL.

---

**References**

1. Bovi JA, White J (2012) Radiation therapy in the prevention of brain metastases. Curr Oncol Rep 14: 55–62.
2. Subramanian A, Harris A, Piggott K, Shieff C, Bradford R (2002) Metastasis to and from the central nervous system—the ‘relatively protected site’. Lancet Oncol 3: 490–507.
3. Nathoo N, Chalhahl A, Barnett GH, Toms SA (2005) Pathobiology of brain metastases. J Clin Pathol 58: 237–242.
4. Gore EM, Bae K, Wong SJ, Sun A, Bonner JA, et al. (2011) Phase III comparison of prophylactic cranial irradiation versus observation in patients with locally advanced non-small-cell lung cancer: primary analysis of radiation therapy oncology group study RTOG 0214. J Clin Oncol 29: 272–278.
5. Gaspar LE, Scott C, Murray K, Curran W (2000) Validation of the RTOG recursive partitioning analysis (RPA) classification for brain metastases. Int J Radiat Oncol Biol Phys 47: 1001–1006.
6. Slotman B, Faivre-Finn C, Kramer G, Rankin E, Snee M, et al. (2007) Prophylactic cranial irradiation in extensive small-cell lung cancer. N Engl J Med 357: 664–672.
7. Umeda T, Tadokoro M, Chen TT, Barkley HT Jr, Bosser DJ, et al. (1984) Role of elective brain irradiation during combined chemoradiotherapy for limited disease non-small cell lung cancer. J Neuroonc 2: 253–259.
8. Cox JD, Stanley K, Fenovitch Z, Paig C, Yesner R (1981) Cranial irradiation in cancer of the lung of all cell types. JAMA 245: 469–472.
9. Russell AH, Pajak TE, Selim HM, Paradelo JC, Murray K, et al. (1991) Prophylactic cranial irradiation for lung cancer patients at high risk for development of cerebral metastasis: results of a prospective randomized trial conducted by the Radiation Therapy Oncology Group. Int J Radiat Oncol Biol Phys 21: 637–643.
10. Cox JD, Scott CB, Byhardt RW, Emami B, Russell AH, et al. (1999) Addition of chemotherapy to radiation therapy alters failure patterns by cell type within non-small cell carcinoma of lung (NSCCL): analysis of radiation therapy oncology group (RTOG) trials. Int J Radiat Oncol Biol Phys 43: 505–509.
11. Ceresoli GL, Reni M, Chiesa G, Garretta A, Schipani S, et al. (2002) Brain metastases in locally advanced nonsmall cell lung carcinoma after multimodality treatment: risk factor analysis. Cancer 95: 603–612.
12. Rohnett TJ, Machay M, Stevenson JP, Algazy KM, Hahn SM (2001) Factors affecting the risk of brain metastases after definitive chemoradiation for locally advanced non-small-cell lung carcinoma. J Clin Oncol 19: 1344–1349.
13. Grunewald-Rashi H, Orik E, Prechtlmann M, Skarda J, Yaron P, et al. (2009) The expression of three genes in primary non-small cell lung cancer is associated with metastatic spread to the brain. Clin Cancer Res 15: 1755–1761.
14. Massague J (2008) TGFbeta in Cancer. Cell 134: 215–230.
15. Javelaud D, Alexsia V, Maesel A (2002) Transforming growth factor-beta in cutaneous melanoma. Pigment Cell Melanoma Res 21: 123–132.
16. Nguyen DX, Bos PD, Massague J (2009) Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 9: 274–284.
17. Meulmeester E, Ten Dijke P (2011) The dynamic roles of TGF-beta in cancer. J Pathol 223: 205–218.
18. Khuri FR, Kim ES, Lee JY, Winn RJ, Benner SF, et al. (2001) The impact of smoking status, disease stage, and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck reirradiation cheomoprevention trial. Cancer Epidemiol Biomarkers Prev 10: 921–929.
19. Yuan X, Xiao Z, Liu Z, Wang L, Tucker SL, et al. (2009) Single nucleotide polymorphism at rs1982073 of the TGFbeta 1 gene is associated with the risk of radiation pneumonitis in patients with non-small-cell lung cancer treated with definitive radiotherapy. J Clin Oncol 27: 3370–3378.
20. Hildebrand MA, Yang H, Hung MC, Izzo JG, Huang M, et al. (2009) Genetic variations in the PTEN/PI3K/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. J Clin Oncol 27: 857–871.
21. Deynck R, Zhang YE, (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425: 577–584.
22. Javelaud D, Alexsia V, Demuler S, Mohammad KS, Guise TA, et al. (2011) TGF-beta/sMASTLGE2 signaling axis in cancer progression and metastasis. Cancer Res 71: 5606–5610.
23. Le Page C, Pauflle ML, Meunier L, Zietarska M, de Ladurantaye M, et al. (2009) BMP-2 signaling in ovarian cancer and its association with poor prognosis. J Ovarian Res 2: 4.
24. Liu M, Stewart DJ, Spitz MR, Hildebrand MA, Lu C, et al. (2011) Genetic variations in the transforming growth factor-beta pathway as predictors of survival in advanced non-small cell lung cancer. Carcinogenesis 32: 1050–1056.
25. McDowell N, Gorden JB (1999) Activin as a morphogen in Xenopus mesoderm induction. Semin Cell Biol 10: 311–317.
26. Huly JR, Chang L, Scholl RR, Widmer HR, Terrell TG, et al. (1994) Induction of apoptosis in the murine liver with recombinant human activin A. Hepatology 20: 854–862.
27. Ball EM, Riesigder GP (2001) Activins as regulators of branching morphogenesis. Dev Biol 238: 1–12.
28. Jones KL, de Kreter DM, Patera S, Phillips DJ (2004) Activin A and follistatin in systemic inflammation. Mol Cell Endocrinol 225: 119–125.
29. de Kreter DM, Hedger MP, Loveland KL, Phillips DJ (2002) Inhibins, activins and follistatin in reproduction. Hum Reprod Update 8: 329–341.
30. Xia Y, Schneyer AL (2009) The biology of activin: recent advances in structure, regulation and function. J Endocrinol 202: 1–12.
31. Butler CM, Gold EJ, Risbridger GP (2005) Should activin betaC be more than a fading snapshot in the activin/TGFbeta family album? Cytokine Growth Factor Rev 16: 377–385.
32. Vejda S, Erdach N, Peter B, Drucker C, Rossmannith W, et al. (2003) Expression of activins C and E induces apoptosis in human and rat hepatoma cells. Carcinogenesis 24: 1801–1809.
33. Mellor SL, Ball EM, O'Connor AE, Ethier JF, Cranfield M, et al. (2003) Activin betaC-subunit heterodimers provide a new mechanism of regulating activin levels in the prostate. Endocrinology 144: 4410–4419.
34. Yin J, Lu K, Lin J, Wu L, Hildebrandt MA, et al. (2011) Genetic variants in TGF-beta pathway are associated with ovarian cancer risk. PLoS One 6: e25559.
35. Auperin A, Arriagada R, Pignon JP, Le Pechoux C, Gregor A, et al. (1999) Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic Cranial Irradiation Overview Collaborative Group. N Engl J Med 341: 476–484.
36. Meert AP, Paesmans M, Berghmans T, Martin B, Mascaux C, et al. (2001) Prophylactic cranial irradiation in small cell lung cancer: a systematic review of the literature with meta-analysis. BMC Cancer 1: 5.
37. Pui CH (2006) Central nervous system disease in acute lymphoblastic leukemia: prophylaxis and treatment. Hematology Am Soc Hematol Educ Program: 142–146.