Table W1. Primers Used for Methylation Analysis.

Gene	Sequence	Product Size (bp)	Annealing Temperature (°C)
p16(CDKN2A)	F: 5′-GGTAATAGTGTTTTTAGAGGTG-3′ R: 5′-CTACCCTAACTAATCTATCTAC-3′	259	60
APC	F: 5′-TAGGGGTTTGAAGGTGTATAGG-3′ R: 5′-CTCCTATAACAAACCTAATCATCAC-3′	305	58
CDH13	F: 5′-TTTATTTGGGAAGTTGGTTGGTTG-3′ R: 5′-TATCCTTCTCAAAATAAACACACAC-3′	442	62
Rassf1	F: 5′-AGGTTGAGATGTTTTTGAGATG-3′ R: 5′-TCCTCCTAACTACAATAACCACTAC-3′	326	59
Nore1A	F: 5′-AGGGTTGGAGATAGAGGTAGAAG-3′ R: 5′-ACAACAACTCCAAAACCTAACC-3′	246	60

Primers used for bisulfite genomic sequencing of the CpG islands of the rat *p16(CDKN2A)*, *APC*, *H-cadherin (CDH13)*, *Rassf1*, and *Nore1A* gene promoters.

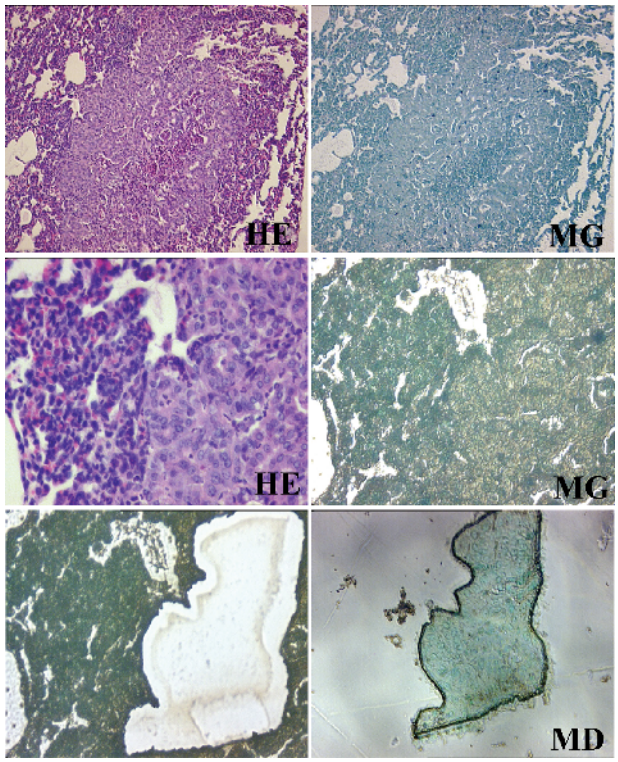

Figure W1. *Microdissection process in SCC mutated in Tp53 (codon 246). One section was stained with H&E, which was used for histologic evaluation and control; another section was stained with methyl green (MG) for microdissection (MD).*

Table W2. Primers Used for Mutational Analysis.

Gene	Exon	Sequence	Product Size (bp)	Annealing Temperature (°C)
Tp53	5	F: 5′-TCCGCTGACCTTTGATTCTT-3′ R: 5′-AGACCCTGGACAACCAGTTC-3′	268	58
	6	F: 5′-CTCCCCGGCCTCTGACTTATT-3′ R: 5′-CCTGGCACACAGCTTCCTAC-3′	274	58
	7	F: 5′-CTTGTGCTGTGCCTCCTCTT-3′ R: 5′-GCCTCCACCTTCTTTGTCCT-3′	198	58
	8	F: 5′-CAAAGTCACCCCTTGCTCTC-3′ R: 5′-CATGCGCTCTGACGATAATG-3′	210	58
	9	F: 5′-TTTGTCCAGCACTTCTGTCCT-3′ R: 5′-CGATGGACATCTGGTGGAGT-3′	250	58
K-ras	1	F: 5′-AGGCCTGCTGAAATGACTG-3′ R: 5′-AGGATGACTGCCACCCTTTA-3′	177	59
	2	F: 5′-TCTCAGGACTCCTACAGGAAAC-3′ R: 5′-GCAGGCCTAACAACTAGCAAA-3′	267	59
N-ras	1	F: 5′-GGTCTGCGGAGTTTGAGATT-3′ R: 5′-CATCCACAAAGTGGTTCTGG-3′	125	57
	2	F: 5′-CCGAAAACAAGTGGTGATTG-3′ R: 5′-ACACACAGAGGAACCCTTCG-3′	125	57
c-H-ras	1	F: 5′-GTTTGGCAACCCCTGTAGAA-3′ R: 5′-TGGGACTCTAACCCATGACC-3′	193	59
	2	F: 5′-AGGGTAGGCGGATTCTCTGT-3′ R: 5′-AGGACTTGGTGTTGTTGATGG-3′	217	59
EGFR	18	F: 5′-GCCCACTCTTGCACTGAATAA-3′ R: 5′-TCCCAGAAGCCTAGTCCAGA-3′	251	58
	19	F: 5′-TAATGCAGAGCCCTTGAGGAT-3′ R: 5′-GGAAACCGTGGTTAGCAAGAC-3′	249	58
	20	F: 5′-CCCATCAGCCAAGAAACAAT-3′ R: 5′-TCCTGCTTCTGAAACCTGCT-3′	303	58
	21	F: 5′-CTGGATGGTTCACTCCCTCA-3′ R: 5′-TCTGGGCTGTCAGGAAAATG-3′	245	58

Primers used for mutational analysis by genomic sequencing of the rat *p53* (*Tp53*), *K-ras*, *N-ras*, *c-H-ras*, and *EGFR* genes.

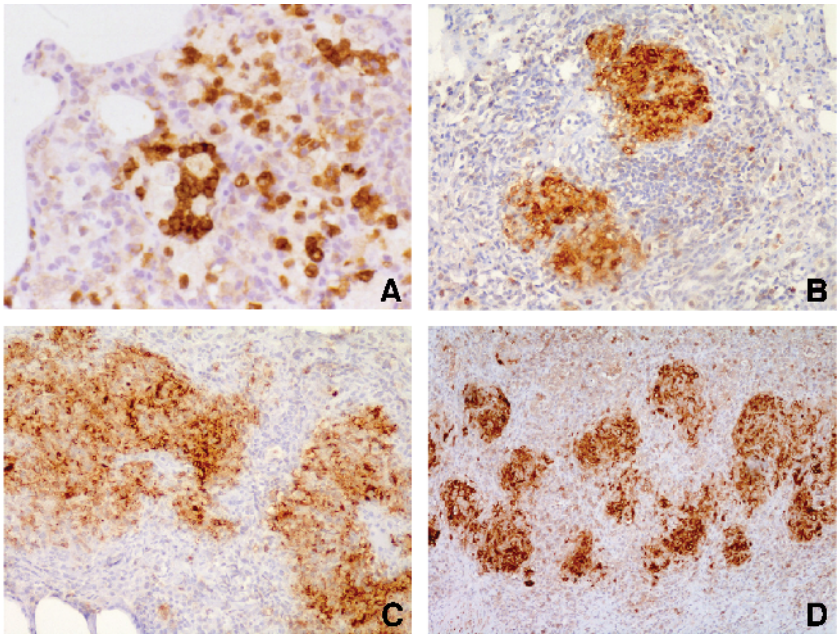

Figure W2. *Immunohistochemical expression of iNOS in macrophages of silica-treated lungs. iNOS was observed in alveolar and parenchymal macrophages of silicotic rats at early stages (A). iNOS expression at the core of granulomas was observed in all the stages of the model (B: month 4; C: month 12; D: month 21). Counterstaining by Harris hematoxylin. Original magnifications, ×140 (D); ×280 (B and C); and ×420 (A).*

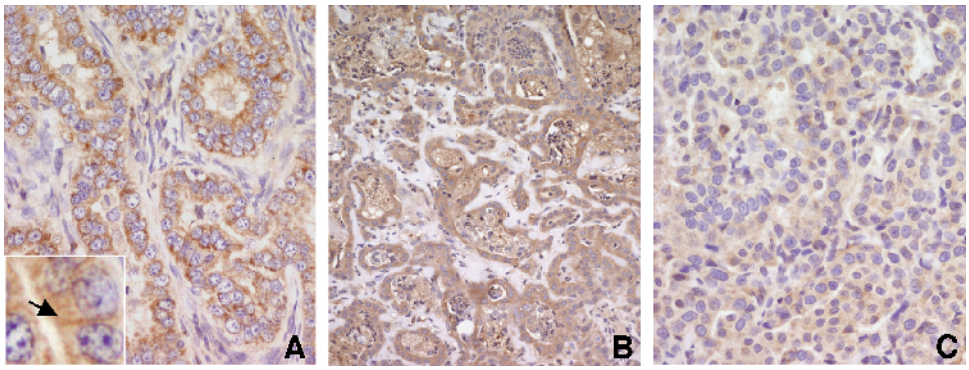

Figure W3. *EGFR expression by immunohistochemistry in silica-induced tumors. Strong or moderate staining of EGFR was found in 55% of AC (A and B) and in 14% of SCC. Only 8% of AC (C) and 29% of SCC were negative. EGFR was localized in the cell membrane and cytoplasm of tumoral cells (arrow; inset, A). Counterstaining by Harris hematoxylin. Original magnifications, ×140 (B); ×420 (A and C); ×920 (inset, A).*

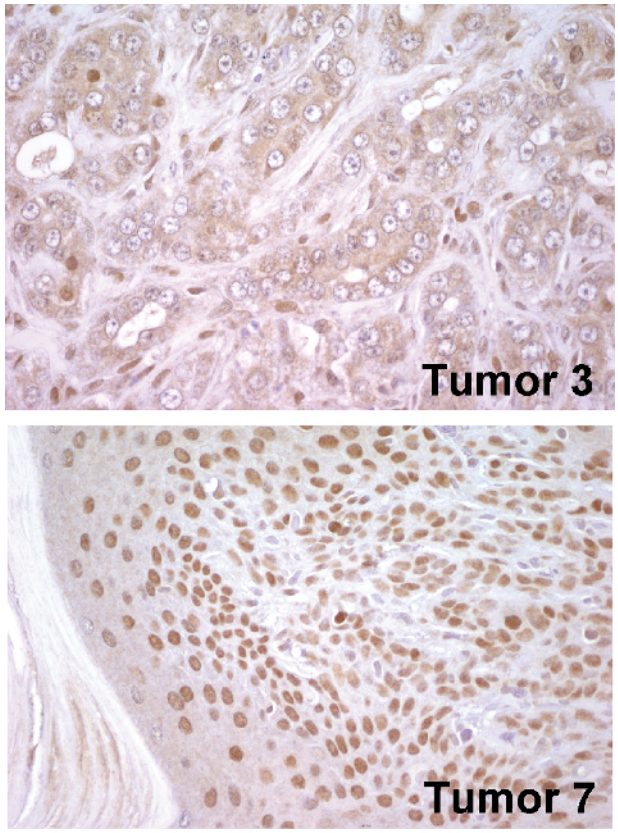

Figure W4. *Direct bisulfite sequencing analyses of the promoter region of p16 revealed a strong correlation between the status of p16 promoter hypermethylation and protein expression in tumors measured by immunohistochemistry. Promoter hypermethylation–positive tumors showed a good association with the loss of nuclear p16 protein expression (tumor 3). This association was also observed in cases without p16 promoter hypermethylation that showed high p16 nuclear protein expression (tumor 7).*

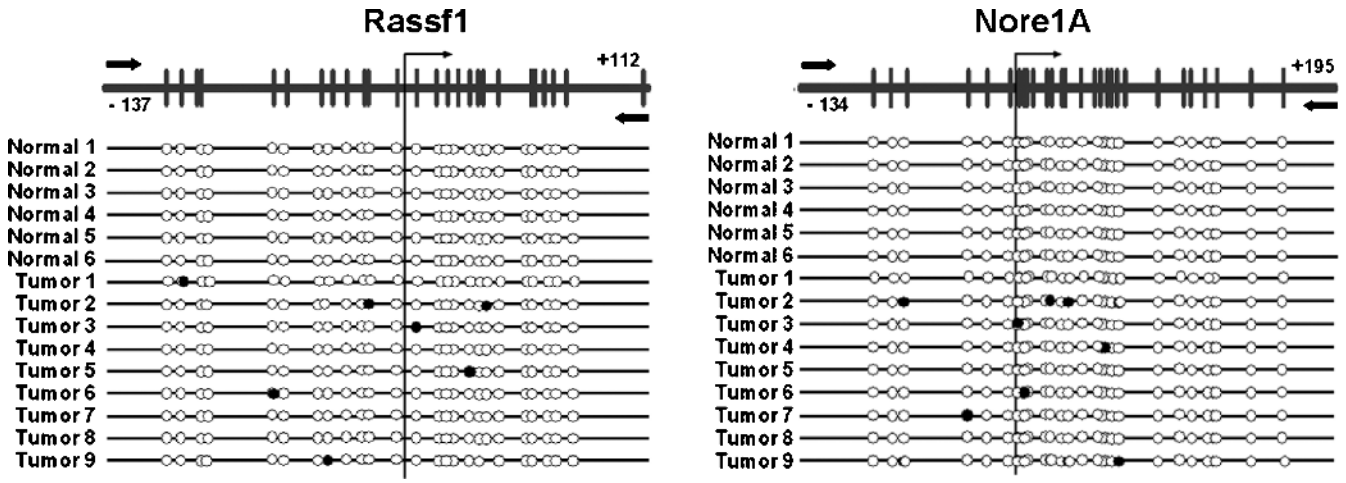

Figure W5. *Bisulfite genomic sequencing of the Rassf1 and Nore1A promoters in nine representative silica-induced lung tumors and six normal tissues. Vertical bars represent the distribution of the CpG islands at the Rassf1 and Nore1A CpG islands. The vertical arrow indicates the transcriptional start point. Black dots indicate methylated CpG islands; white dots indicate unmethylated CpG islands. The position of the bisulfite sequencing primers used is represented with white horizontal arrows. We did not observe significant Ras effectors promoter hypermethylation in any of the tumors analyzed.*