

**p16INK4A inactivation mechanisms in non-small-cell lung cancer patients occupationally exposed to asbestos.**

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## LEGEND TO FIGURES

### **Figure 1. Immunodetection of p16<sup>INK4A</sup> by immunohistochemistry in NSCLC patients**

A: Positive control of p16<sup>INK4A</sup>

B: Positive immunostaining of p16<sup>INK4A</sup> in a lung adenocarcinoma case (× 200)

C: Lack of p16<sup>INK4A</sup> expression in a lung adenocarcinoma case (× 200)

### **Figure 2. Representative patterns of aberrant methylation promoter of *P16/CDKN2A* gene in NSCLC patients.**

Primer sets used for amplification are designated as unmethylated (UM) or methylated (M). Twenty µl of PCR product were run on 2.5% agarose gel stained with ethidium bromide, and visualized under UV illumination. T: tumor tissue; N: normal lung tissue; M: DNA 50 bp ladder; N<sub>M</sub>: negative control with methylated sequence specific primer; N<sub>UM</sub> negative control with unmethylated sequence specific primer.

**Figure 3. FISH hybridization.** Dual-color FISH with PONC0921 probe. (*P16/CDKN2A* specific DNA probe is direct-labeled with rhodamine and the chromosome 9 classical satellite probe is direct-labeled with fluorescein).

**A:** Normal lung tissue used as positive control showed two red signals and two green signals.

**B:** NSCLC case showing two green signals and one red signal indicating a loss of heterozygosity of *P16/CDKN2A* gene.

**C:** NSCLC case showing two green signals and no red signal, indicating a

homozygous deletion of *P16/CDKN2A* gene.