I S—11 Analysis of oncosuppressor gene p53, Rb and CDK4 inhibitor gene (p16) in human ovarian carcinoma

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We analyzed oncosuppressor gene p53, Rb and CDK4 inhibitor gene p16 by using molecular biology techneques in human ovarian carcinoma tissues and cell lines (PA-1, Caov3, Caov4, SK-OV-3, OVCAR-3, Kuramochi). P16 gene inhibit the phosphorylation of Rb protein. Southern blot. analysis of p53 gene revealed gene rearrangement in SK-OV-3. Northern blot. and RT-PCR showed no mRNA of p53 was expressed in SK-OV-3. We couldn't detect p53 protein in Caov-3 and SK-OV-3 by immunoprecipitation. Sequencing of p53 cDNA revealed point mutation or minor deletion in PA-1, Caov-3, Caov-4, OVCAR-3, and Kuramochi. In Pa-1 cell line point mutation is nonsense mutation that resulted no amino acid change. Southern blot. analysis of Rb gene showed no abnormalities in all cell lines. Southern blot. and RT-PCR revealed no expression of Rb mRNA in Caov-3. We detected a point mutation inCaov-4 by sequencing analysis of Rb cDNA that result amino acid change og Rb protein. CDK4 inhibitor gene P16 analysis of exon 2 showed deletion or gene rearrangement in SK-OV-3. PCR-SSCP analysis of p16 exon2 revealed no abnormalities in other cell lines. In analysis of human ovarian carcinoma tissues, we extracted DNA from paraffin-embedded tissues. P53 gene showed 20 % loss of heterozygosity (LOH) by PCR-LOH analysis, and 15% in Rb gene. From these results we speculate that abnormalities of oncosuppressor gene p53, Rb gene and CDK4 inhibitor gene p16 play a important role in human ovarian carcinogenesis.

I S—12 Mutations and altered expression of $p16^{INK4}$ in human cancer.

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$p16^{INK4}$ was identified as an inhibitor of activated cyclin D-cdk4 complex. Expression of $p16^{INK4}$ and alteration of this gene were analyzed in 34 human tumor cell lines including 5 ovarian tumor cell lines. $p16^{INK4}$ mRNA and protein were not detected in 12 of 28 (43%) and 27 of 34 (79%) tumor cell lines, respectively, whereas normal WI38 human fibroblasts and the nontumorigenic SV-40 T antigen immortalized human cell lines showed $p16^{INK4}$ mRNA and protein. The presence of $p16^{INK4}$ protein is inversely correlated with detectable Rb or cyclin D1 proteins and is not correlated with p53 mutations, indicating that mutations in either Rb or $p16^{INK4}$ is sufficient to disrupt the G1 checkpoint pathway and that p53 participates in an independent pathway. Southern blot, PCR-SSCP, sequencing analysis detected 6 homozygous deletions, 1 rearrangement and 6 mutations in these cell lines (43%). We also examined alterations of this gene in 188 human primary tumors including 26 ovarian tumors. Although 4 homozygous deletions and 2 mutations were detected in 22 lung metastases (27%), primary lung, ovarian and other tumors showed infrequent alterations. These results suggest that $p16^{INK4}$ is a tumor suppressor gene and it may play a role as a late event in human carcinogenesis.