

A CHARACTERIZATION OF THE TEMPERATURE-SENSITIVITY OF AN OVARIAN CARCINOMA CELL LINE (OVBH-1), INDEPENDENT OF P53 STATUS

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OvBH-1 cells from a patient with ovarian clear cell carcinoma were established and their biochemical status was analysed. Cells grown at 37°C exhibited normal cell cycle distribution, whereas the cells shifted to 31°C were arrested in the G₂/M phase of the cell cycle. Immunohistochemical analysis using anti-p53 antibodies (DO-1, PAb240, PAb421, and PAb1620) revealed that only the DO-1 antibody reacted with p53 to a high and similar level (percentage) at both temperatures. PAb240 reacted to a low level (percentage) at 37°C, and no reaction was observed at 31°C. The PAb421 antibody stained a significantly lower percentage of cells at 37°C than at 31°C. The cells did not stain with the PAb1620 antibody, and were negative for antibodies against the p21^{WAF1} and MDM2 proteins independently of temperature. The sequencing of all the coding exons of the *p53* gene demonstrated only a neutral genetic polymorphism, i.e. a G to A substitution (GAG to GAA) at nucleotide position 13432. Thus, the observed temperature sensitivity of OvBH-1 cells cannot be ascribed to a p53 primary structure mutation. Based on immunochemical analyses, we conclude that the p53 in the nuclei of OvBH-1 cells appears to be in a highly unstable conformation. Furthermore, the N-terminal portion of the p53 protein at Ser20 has not been modified, and the Lys373 and/or Ser378 of the C-terminus is acetylated and/or phosphorylated. The nuclear location signal of p53 is preserved. Induction of the MDM2 protein is uncoupled from the cell regulatory machinery and the induction of p21^{WAF1} by p53 is impaired in OvBH-1 cells.