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 p21WAF1 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 p21WAF1 by p53 is impaired in OvBH-1 cells.