TP53 domains' mutations alter glycolysis in epithelial ovarian carcinoma: ex-vivo and in vitro study

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BACKGROUND/AIM

To investigate the effect of TP53 different domain mutations on its transcriptional activity, its ability to induce apoptosis and to regulate glucose consumption and lactate production in epithelial ovarian cancer.

METHODS

30 ovarian cancer biopsies were extracted and characterized by Hôtel-Dieu de France University Hospital – Lebanon. Immunohistochemistry for the expression of p53 was performed on fresh frozen sections. In order to detect and study the mutations affecting TP53 gene, DNA was extracted directly from the tissues, and PCR for exons 2 till 11 was performed, followed by the Single Strand Conformation Polymorphism (SSCP) technique and sequencing. 6 of these cases were cultured (2 wild type and 4 mutant TP53), and the transcriptional activity of p53 was studied by a qPCR for its target genes p21 and MDM2.

Viability and Annexin V tests were performed to study the ability of mutant p53 to induce apoptosis. Cell culture medium was collected, lactate concentration was measured by spectrophotometry, and glucose consumption by the glucose oxidase activity assay. In addition, the expression of the glycolytic enzymes regulated by p53 was quantified by qPCR. SK-OV-3 cell line was transfected by different p53 mutated plasmids, and the same experiments performed on the biopsies were done on transfected cells.

RESULTS

- 13 out of the 30 cases were characterized as p53 mutant ovarian carcinomas: 6 patients showed mutations affecting the apoptosis domain of the gene (exon 2-4), 3 with mutations in the DNA binding domain (exon 5-8), and 4 with mutations in both domains. The immunohistochemistry and qPCR results showed an approximately 2 folds increase in p53 expression between wild type and mutated cases.

FIGURE 1. Effect of the mutation sites on p53 expression and transcriptional activity.

FIGURE 2. Effect of the different domain mutations on glucose consumption and lactate production.

FIGURE 3. Effect of p53 site mutations on apoptosis initiation. The influence of the mutations affecting the various domains of the p53 gene on apoptosis was assessed in the transfected SK-OV-3 cells. After transfection for 24 and 48 h, the cells were harvested and Annexin V was performed by flow cytometry.

- The expression of p21 and MDM2 decreased only in DNA binding domain mutated cases and transfected cells, which indicates a decrease in the transcriptional activity with this type of mutation. The highest increase in apoptosis induction was clear in Sk-Ov-3 cells transfected with WT p53, and p53 proline rich domain mutations decreased the protein's apoptotic function.
- Glucose consumption and lactate production increased by the mutated cells compared to the wild type, which correlates with the alteration in the glycolytic enzymes' expression.

CONCLUSION

Mutant p53 is overexpressed in ovarian cancer cells. The alteration in the activity of the mutant protein depends on the locus of the mutation. DNA binding domain mutations modify the protein's transcriptional activity, whereas proline rich domain mutations decrease the protein's apoptotic activity. Glycolysis is affected differently in both types.