Review Article

Proteins P53 and B-cell lymphoma-2 in pathogenesis missed and spontaneous abortions

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ABSTRACT

mRNA expression of p53, p21, B-cell lymphoma-2 (BCL-2), Bax, Noxa and MDM2 in decidual tissue (endometrium) of 50 patients with missed abortions, 47 patients with spontaneous abortions, and 57 patients with artificial abortion (control group) at 6–10 weeks of gestation were examined using reverse transcription quantitative polymerase chain reaction. Expression of 53 mRNA had no significant differences in patients with missed and spontaneous abortions. Significant increase of proapoptotic protein Noxa, accompanied with anti-apoptotic protein BCL-2 elevation was observed in the endometrium of patients with missed abortions. In patients with spontaneous abortions, only increase of Noxa has been observed. It can be suggested, that increase of BCL-2 in patients with missed abortions, when embryo resorption has happened, protects nearby embryos in case of multiple pregnancies. No significant changes of p21 mRNA were discovered in both groups, which indicates, that cell cycle arrest is not involved in the pathogenesis of early miscarriages. No significant changes of Bax and MDM2 mRNA expression were discovered in decidual tissue of patients with missed and spontaneous abortions.

KEY WORDS: Apoptosis, Bax, B-cell lymphoma-2, Decidual tissue, Endometrium, MDM2, Missed abortion, Noxa, p21, p53, Spontaneous abortion`

INTRODUCTION

Missed and spontaneous abortions are one of the main causes of reproductive losses.[1,2] Except for endocrine disorders and antiphospholipid syndrome, miscarriages can have infection origin, which may induce proinflammatory cytokines production and final result to prostaglandins synthesis.[3] There are some recent data that apoptosis of different origin may also play an important role in the pathogenesis of early miscarriages.[4,5]

One of the main inducers of intrinsic pathway of apoptosis protein is p53, also known as tumor protein p53 (Tp53), or tumor suppressor gene. P53 has multiple actions in the cell. It can activate DNA repair proteins, arrest growth of cell in case of recognized DNA damage and initiate apoptosis, if DNA damage proves to be irreparable [Figure 1].[6]

Activation of apoptosis appears through the Bax and Noxa pathways. Protein p21 (CDKN1A) mediates p53-dependent G1 growth arrest. If it holds the cell in growth arrest for long enough, the DNA repair proteins will have time to fix the damage, and the cell will be allowed to continue the cell cycle.

The main suppressor of p53 is protein MDM2, which inhibit it by binding, preventing its action and transports it from the nucleus to the cytosol. Therefore, MDM2 is known as an oncogene, but it also provides reepithelialization on epithelial damage.[7] Proapoptotic protein Bax can be inhibited by anti-apoptotic protein B-cell lymphoma-2 (BCL-2).[8]

However, the role of p53 and proteins of its signaling pathway in pathogenesis of missed and spontaneous abortions are not well studied.

Objective

The objective of the study was to estimate features of p53 and its signal proteins mRNA expression in patients with missed and spontaneous abortions at 6–10 weeks of gestation.

MATERIALS AND METHODS

The main group included 50 patients with missed abortions and 47 patients with spontaneous abortions,

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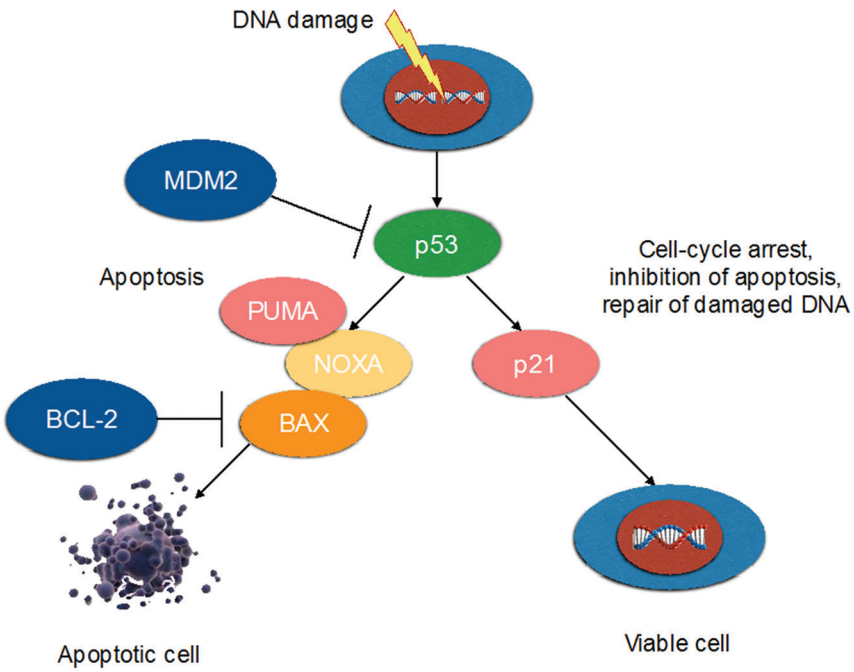

Figure 1: Pathway of p53 protein (Courtesy: Rowland and Peeper, in modification)[6]

admitted to Belgorod Municipal City Clinical Hospital No. 1 at 6–10 weeks of gestation; the control group included 57 women who applied for medical abortion at the same stage of pregnancy. Patients with severe extragenital diseases, endocrine disorders and antiphospholipid syndrome were excluded from the research.

Epithelial cells were obtained by uterine abrasion and placed into preservative solution RNA later ("Ambion," USA). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to identify mRNA expression of p53, p21 (CDKN1A), Noxa, Bax, BCL-2 and MDM2 according to MIQE guideline.[9] RNA was obtained by phenol-chloroform extraction using reagent "Trisol" ("Invitrogen," USA). RNA quality was assessed through 15 min. electrophoresis at 10 V/cm based on the intensity of fluorescence of ribosomal RNA bands in 1.1% agarose gel with ethidium bromide. The obtained RNA was treated with DNAse using DNAse I RNAse free kit ("Thermo Fisher Scientific," USA). To perform RT, a reverse transcriptase Mint kit and oligo DT ("Evrogen," Russia) were used. 500 ng of RNA were added to the mixture for the reaction. RT was made according to manufacturer's instruction in amplifier "Tercyc" ("DNA-technology," Russia). Resulting cDNA quality was assessed using electrophoresis in 1.1% agarose gel with ethidium bromide. Resulting cDNA with sterile water (1:25) was used for the RT-qPCR. specific primers for PCR were selected in database BLAST (www.ncbi.nlm.nih.gov) [Table 1]. Peptidylprolyl isomerase A and β-actin were used as housekeeping genes. Amplification was carried out in amplifier CFX96 ("Bio-Rad," USA) using qPCR-mix HS SYBR kit ("Evrogen," Russia). Amplification was performed using the following cycling conditions: 5 min at 95°C, and 45 three-step cycles of 15 s at 95°C, 30 s of appropriate gene annealing according to Table 1 and 30 s at 68°C.

The obtained results were expressed in relative units, which were calculated by the formula:

$$R = 2^{-(Cq\ target - (Cq\ ref_1 + Cq\ ref_2)/2)},$$

Where R is a normalized mRNA expression of genes under investigation; Cq ref_1, and Cq ref_2–Cq housekeeping genes; Cq target–Cq of the gene under investigation.[10]

Statistical processing of the data obtained was carried out using Statistica 13.2 (StatSoft, USA). The significance of differences was evaluated according to Mann–Whitney test, the results of which were represented as a median (lower quartile; upper quartile). The Spearman's rank criterion was used for the correlation analysis. The results were considered as significant, if $P \leq 0.05$.

RESULTS AND DISCUSSION

It was established, that p53 mRNA expression level in patients with missed and spontaneous abortion had no significant differences with the control group (progressive pregnancy) [Table 2]. mRNA expression of MDM2, which is a negative regulator of p53, also did not have significant differences in both groups.

Expression of Bax mRNA did not have significant changes in the endometrium of patients with missed and

Table 1: Primers for RT-qPCR

Gene	Forward primer 5'-3'	Reverse primer 5'-3'	Annealing temperature, °C
p53	AGCTGTGGGTTGATTCCACAC	TTTCTTCTTTGGCTGGGGA	58
p21	TGGGGATGTCCGTCAGAA	TTCCTCTTGGAGAAGATCAGC	55
Noxa	CCAAACTCTTCTGCTCAGGAA	ATCACAGGTCATCTCCCTTCA	63
Bax	CGCCCTTTTCTACTTTGCCAG	TGGAGACAGGGACATCAGTCG	55
BCL-2	CGTTTGGCAGTGCAATGGT	TTCTTGATTGAGCGAGCCTT	63
MDM2	TTCGTGAGAATTGGCTTC C	GGCAGGGCTTATTCCTTTTCT	61
β-actin	CAGGCACCAGGGCGTGATGG	GATGGAGGGGCCGGACTCGT	64
PPIA	CCGCCGAGGAAAACCGTGTACT	TGGACAAGATGCCAGGACCCGT	64

BCL-2: B-cell lymphoma-2, PPIA: Peptidylprolyl isomerase A, RT-qPCR: Reverse transcription quantitative polymerase chain reaction

Table 2: Expression of p53 and signaling pathway proteins mRNA in endometrium of patients with missed and spontaneous abortions and the control group, Me (25%; 75%), relative units

Gene	Patients with missed abortion (*n*=50)	Patients with missed abortion (*n*=47)	Control group (*n*=57)	P 1–3	P 2–3	P 1–2
p53	0.163 (0.052; 0.195)	0.215 (0.038; 0.430)	0.162 (0.028; 0.273)	0.15	0.34	0.38
p21	0.00014 (0.00009; 0.00719)	0.00008 (0.000001; 0.00155)	0.00015 (0.000002; 0.00092)	0.13	0.85	0.16
Noxa	0.0442 (0.0077; 0.0822)	0.01448 (0.00036; 0.0810)	0.00001 (0. 000001; 0.0003)	0.003	0.02	0.59
Bax	0.00199 (0.000007; 0.0248)	0.0007 (0.00002; 0.0102)	0.00007 (0.000001; 0.0126)	0.07	0.20	0.55
BCL-2	0.00113 (0.00004; 0.1187)	0.00005 (0.000013; 0.0088)	0.00009 (0.000008; 0.000044)	0.02	0.63	0.28
MDM2	0.0060 (0.00009; 0.0071)	0.0030 (0.00001; 0.0356)	0.0068 (0.0001; 0.0231)	0.56	0.06	0.61

BCL-2: B-cell lymphoma-2

spontaneous abortions. However, mRNA of proapoptotic protein Noxa was drastically increased in decidua of patients with missed abortions. It was accompanied by an increase of proapoptotic protein BCL-2 mRNA. In decidual tissue of patients with spontaneous abortions, only increase of Noxa mRNA was observed.

Expression of p21 (CDKN1A) mRNA in both groups (missed and spontaneous abortions) did not have significant differences with the control group, which indicates, that G1 cell cycle arrest is not involved in the pathogenesis of early miscarriages.

The research data about the level of p53 and proteins of its pathway in trophoblast and endometrium of patients with miscarriages are contradictory. In the research of Pang *et al*. decreasing of BCL-2 and increasing of Bax in trophoblast were observed.[11] However, study group included missed and spontaneous abortions both, without their division into subgroups, in spite that pathogenesis of these conditions is different. In another research by immunohistochemical method, it was shown, that level of BCL-2 is decreased, and level of p53 is increased in patients with recurrent miscarriages.[12] However, in this study, the control group consisted of patients with sporadic spontaneous abortions. Therefore, this group could not be considered as normal (progressive pregnancy). Increased level of p53 and decreased level of BCL-2 were observed in the study of Gurevich *et al.*[13] Meanwhile, in this research study group was not divided into missed and spontaneous abortions, control group contained patients with tubal abortions.[13]

On the contrary, in the study of Wang *et al.* by Western-blotting, it was revealed, that BCL-2 was significantly up-regulated in the trophoblast villi of recurrent miscarriage patients.[14] In cases of missed abortions increase of p53, Bax and p21 (CDKN1A) were observed (Shang *et al.*, 2013).[15] Upregulation of p53 mRNA and protein in chorional so was found in patients with recurrent pregnancy loss.[16] Interestingly, p53 protein expression was significantly higher in patients with recurrent pregnancy loss compared with sporadic abortions and control group.[17] It is necessary to emphasize that in all above-mentioned reports trophoblast villi were taken for the study. Decidual tissue was not taken for the research.

In endometrium (decidual tissue) of mice having embryo resorption a significant upregulation of the anti-apoptotic BCL-2 protein was found, while no changes were observed for proapoptotic molecules.[18] Authors did not find any significant changes in BCL-2 expression of trophoblast. It is known, that level of BCL-2 in endometrium increases in case of embryo resorption.[19] Therefore, it can be suggested, BCL-2 increase in the endometrium in case of missed abortion and embryo resorption may reflect a compensatory mechanism tending to protect and maintain safely of nearby embryos in case of multiple pregnancies.[18] On the contrary, in another study of patients with spontaneous miscarriages expression of p53 in decidual tissue was increased, while expression of BCL-2 – reduced.[20] Expression levels of TP53 protein, p21 (CDKN1A), and Bax mRNA in decidual tissue were found to be significantly increased in patients with recurrent miscarriages.[21]

Concerning MDM2 as a regulator of p53 activity, it was mostly shown, that its gene polymorphisms are associated with recurrent pregnancy loss.[22,23] There

are no data available in the literature concerning the role of protein Noxa in the pathogenesis of missed and spontaneous abortions.

Therefore, it could be suggested, that significant increase of BCL-2 in patients with missed abortion can be related to embryo resorption, may be compensatory mechanism of protection of nearby embryos in case of multiple pregnancies. This can explain why BCL-2 was not elevated in cases of spontaneous abortions when embryo stays alive until full detachment of gestational sac from uterine wall.

Increased expression of Noxa in decidual tissue of patients with missed and spontaneous abortions suggests its important role in pathogenesis of all cases of early miscarriages. As Noxa transcription can be only induced by p53,[24,25] further research of p53 expression on the level of protein in endometrium should be done to prove this conception.

CONCLUSION

Thus, sufficient increase of proapoptotic protein Noxa, accompanied with anti-apoptotic protein BCL-2 elevation was observed in the endometrium of patients with missed abortions. In patients with spontaneous abortions, only increase of Noxa has been observed. It can be suggested, that increase of BCL-2 in patients with missed abortions, when embryo resorption has happened, protects nearby embryos in case of multiple pregnancies. No significant changes of p21 mRNA were discovered, which indicates, that cell cycle arrest is not involved in the pathogenesis of early miscarriages.

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