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SHORT COMMUNICATIONS

Interaction between Hsp60 and Bax in normal human myocardium and in myocardium affected by dilated cardiomyopathy

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The main functional compartments of molecular chaperone Hsp60 are mitochondria and cytoplasm. Up to 30 % of Hsp60 are located in cytoplasm of cardiomyocytes. The interaction between molecular chaperone Hsp60 and proapoptotic Bax protein in the cytoplasmic fraction from normal human heart tissue has been revealed by co-immunoprecipitation in contrast to myocardium affected by dilated cardiomyopathy, where this interaction has not been observed.

Keywords: Hsp60, Bax, myocardium, dilated cardiomyopathy.

Introduction. Molecular chaperones are known as cardioprotective proteins, playing a role of key regulators of apoptosis or survival of cardiomyocytes [1]. Molecular chaperone Hsp60, the main functional compartments of which are mitochondria, is of special interest, since the recent data evidence to the location of 10-30% of this protein in cytoplasm of cardiomyocytes [2].

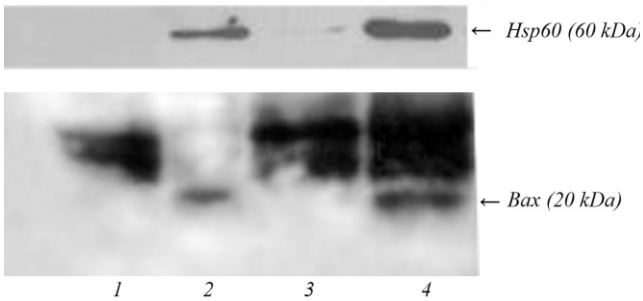
Our previous experiments proved the decrease in Hsp60 content in cytoplasmic fraction of cardiomyocytes at dilated cardiomyopathy (DCM) progression. DCM is a cardiac pathology causing a loss of cardiomyocytes due to apoptosis [3].

A number of investigations on cultured neonatal rat cardiomyocytes [2, 4-6] demonstrated that Hsp60 is capable to form the complexes with proapoptotic proteins Bax and Bak preventing the rise of apoptosis. The decrease in Hsp60 level in cardiomyocytes resulted in translocation of Bax protein to mitochondria and launch of apoptosis.

It is noteworthy that cultured cardiomyocytes are quite different from cardiomyocytes of native heart tissue in their ultrastructure and functional features. At present, there are no data on existence of Hsp60 complexes with proapoptotic proteins of Bcl-2 group, Bax type, in intact myocardium.

Our research was aimed at revealing a possible interaction of Hsp60 with proapoptotic Bax protein in cytoplasmic fractions of cardiomyocytes, obtained from normal human heart tissue and myocardium, affected by dilated cardiomyopathy.

Materials and Methods. Monoclonal anti-Bax antibodies were purchased from *Santa Cruz*, USA. The methods of development, purification, and characterization of anti-Hsp60 antibodies have been previously described [7]. An extract of cytoplasmic



Western blot analysis: test for non-specific binding of Bax and Hsp60 to protein-G-Sepharose (*1*); cytoplasmic fraction of cardiomyocytes, obtained from normal myocardium (*2*); immunoprecipitation of cytoplasmic fractions, obtained from myocardium, affected by dilated cardiomyopathy (*3*), and from normal human heart tissue (*4*), using anti-Bax (upper panel) and anti-Hsp60 antibodies (lower panel)

fraction of cardiomyocytes was obtained from normal human heart tissue and myocardium, affected by DCM, using the method previously described [3]. Immunoprecipitation was performed according to [8]. Cytoplasmic fractions (1 mg/ml) were preabsorbed with 20 µl of protein-G-Sepharose beads (*Sigma*, USA) at 4° for 30 min, centrifuged (5 min at 10 000xg), the supernatant was incubated with corresponding antibodies (5 µg of antibodies for 1 mg of total protein) at 4°C overnight. Then 20 µl of proteinG-Sepharose beads were added and incubation was continued for 1.5 hour at 4°C. Immunocomplexes were collected by centrifugation and washed 4 times with cold buffer (137 mM NaCl, 20 mM tris-HCl, pH 7.5, 1% triton X-100, 2 mM EDTA, pH 8.0, 2 mM PMSF). The final products were briefly boiled and analyzed by SDS-PAGE and Western-blotting with specific antibodies as indicated [3].

Results and Discussion. Immunoprecipitation of a possible complex of Hsp60/Bax from the cytoplasmic fraction obtained from normal human heart tissue and DCM-affected myocardium was performed using anti-Hsp60 and anti-Bax antibodies. The complex was identified by Western-blot analysis. The results are presented in Figure 1. It shows that Hsp60 forms a complex with proapoptotic protein Bax in normal human heart tissue. However, this complex has not been revealed in the myocardium affected by DCM. It testifies to the absence or decrease of the Hsp60-Bax complex in cytoplasm of DCM-cardiomyocytes to the amount that is beyond the detection by antibodies. The latter may be explained by decrease in the amount of one or both of these proteins in cytoplasmic fraction. As mentioned above, we have shown the decrease in the amount of the cytoplasmic Hsp60 at dilated cardiomyopathy [3].

There is no information on the level of Bax protein in cytoplasm of cardiomyocytes at DCM, however, some literature data show that this disease does not cause any decrease in the total amount of this protein in the heart tissue at heart failure progression. Some authors state an increase in the amount of Bax protein [9], others did not find any significant difference compared to the normal myocardium [10]. Therefore, we think it is possible to assume that the decrease in the amount of cytoplasmic Hsp60 at DCM causes the increase in the level of Bax, "free" from Hsp60, in cytoplasm resulting in the same consequences described for the cultured cells of neonatal cardiomyocytes at stress conditions, i.e. in translocation of Bax to mitochondrial membrane and launch of programmed cell death.

The current work proves the existence of the complex between endogenous Hsp60 and Bax protein in normal human heart tissue analogous to the one identified previously in neonatal rat cardiomyocytes [6]. This interaction has not been observed in DCM-affected cardiomyocytes.

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Взаємодія між ендогенними Hsp60 та Bax у нормальному та ураженому дилятаційною кардіоміопатією міокардах людини

Резюме

Hsp60 – молекулярний шаперон, основними компартментами функціонування якого є мітохондрії та цитоплазма. В кардіоміоцитах до 30 % цього білка знаходиться у цитоплазмі. Методом коімунопреципітації виявлено взаємодію між молекулярним шапероном Hsp60 та проапоптичним білком Bax у цитоплазматичній фракції кардіоміоцитів, отриманій із нормальної тканини серця людини, на відміну від кардіоміоцитів, уражених дилятаційною кардіоміопатією, де такої взаємодії не спостерігається.

Ключові слова: Hsp60, Bax, міокард, дилятаційна кардіоміопатія.

Л. Н. Капустян, О. Т. Рожко, И. А. Тихонкова, Л. Л. Сидорик

Взаимодействие между эндогенными Hsp60 и Bax в нормальном и пораженном дилятационной кардиомиопатией миокардах человека

Резюме

Hsp60 – молекулярный шаперон, основными компартментами функционирования которого являются митохондрии и цитоплазма. В кардиомиоцитах до 30 % этого белка находится в цитоплазме. Методом коиммунопреципитации обнаружено взаимодействие между молекулярным шапероном Hsp60 и проапоптическим белком Bax в цитоплазматической фракции кардиомиоцитов, полученной из нормальной ткани сердца человека, в отличие от кардиомиоцитов, пораженных дилятационной кардиомиопатией, где такого взаимодействия не наблюдалось.

Ключевые слова: Hsp60, Bax, миокард, дилятационная кардиомиопатия.

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