THE FAS-RELATED APOPTOSIS SIGNALING PATHWAY IN THE PROSTATE INTRAEPITHELIAL NEOPLASIA AND CANCER LESIONS

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Abstract: An aim of the study was to determine the protein expression of the FAS-related apoptosis signaling pathway (FADD-FAS Associating Protein with Death Domain, PRO-CASPASE-8 and CASPASE-8), which are responsible for signal transduction to trigger programmed cell death (apoptosis) in cancer and Prostatic Intraepithelial Neoplasia (PIN). 20 specimens from prostate cancer patients treated with radical prostatectomy were investigated. 8 cancers were diagnosed as G-2 and 12 as G-3. 14 samples were described as poorly differentiated, high Gleason score (≥ 7). Control group consisted of prostate specimens from autopsy of 3 young men. Specimens were fixed in 10% buffered formaldehyde and immersed in paraffin. Haematoxylin and eosin staining was done. Monoclonal antibodies to FADD & CASPASE-8 (Novocastra, UK) were used to immunohistochemical study, according to streptavidine-biotin method. Semiquantitive method described protein expression. Expression index (EI) was calculated as a percent of positive FADD or CASPASE-8 cells to total cells in the specimen. Statistical analysis was performed with the Student t-test (p < 0.05). Normal prostate tissue was negative in both, FADD and CASPASE-8 immunohistochemistry staining. PIN & prostate cancer lesions were found to strongly express of FADD & CASPASE-8 proteins. Expression of FADD in cancer lesions was 66,5 ± 27,8% and 59,8 ± 19,0% vs. 56,8 ± 14,8% HGPIN and LGPIN, respectively. Expression of CASPASE-8 in cancer lesions was 64,1 ± 23,4% and 61,5 ± 15,0% vs. 48,0 ± 17,6% HGPIN and LGPIN, respectively. PIN & prostate cancer lesions are characterized by similar high expression of proteins responsible for signal transduction to induce apoptosis. The mediators of apoptotic signal can be very important in prostate cancer prophylaxis and management.

Keywords: apoptosis, Tumor Necrosis Factor, FAS, FAS Associated Death Domain, prostate cancer, Prostate Intraepithelial Neoplasia

Induction of the apoptosis – Programmed Cell Death (PCD) can be the key for prostate cancer treatment. FAS (CD95, Apo-1) and TNFR-1 membrane receptors are responsible for apoptosis triggering in the prostate epithelium. Those receptors belong to Tumor Necrosis Factor (TNF) family.

The FAS (CD95, Apo-1) receptor occurs in the cell membrane. After binding with a ligand (FASL), the receptor FAS undergoes trimerisation, which consists in joining three identical receptor/ligand units. On the cytoplasm side, FAS possesses a sequence called the Death Domain – DD. The stimulation of the membranous receptor FAS is transmitted through the intermediary particle FAS Associating Protein with Death Domain (FADD), also called Mediator of Receptor-induced Toxicity 1 (MORT1), which also possesses a DD. Through DD the receptor links with the matching DD on the intermediary particle FADD. Apart from DD, the FADD particle possesses a segment called the Death Effector Domain – DED. Thanks to the DED sequence, FADD protein activates PRO-CASPASE-8 and also CASPASE proenzymes 7 and 9 (1,2). The activation of these proenzymes provokes an avalanche reaction of caspases, which leads to the executive phase of apoptosis and cell death. The 7 and 9 CASPASES activation can be modulated through TNFR-1 (Tumor Necrosis Factor Receptor-1) related surviving signals. Janig et al. observed increased FAS and its ligand (FASL) expression in the prostate cancer cells and Prostate Intraepithelial Neoplasia (PIN) lesions, which are premalignant lesions (3).

Previous studies on the expression of receptors related pathways responsible for apoptosis triggering were conducted on animals and cell lines.

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The estimation of the FADD and CASPASE-8 expressions in human prostate epithelial cells may elucidate important topics concerning prostate cancer management and prevention (4).

The aim of this study was to determine the protein expression of the FAS-related apoptosis signaling pathway (FADD, PRO-CASPASE-8 and CASPASE-8), which are responsible for signal transduction to trigger receptor related programmed cell death in cancer and PIN cells.

EXPERIMENTAL

20 specimens were from prostate cancer patients treated with radical prostatectomy. 8 cancers were diagnosed as G-2 and 12 as G-3. 14 cancers were described as poorly differentiated according to Gleason score (Gl-s 7 and more). Control group consisted of prostate specimens from autopsy of 3 young men aged 40 or less, who died unexpectedly. During histopathological examination of surgically removed prostate glands Low-grade Prostatic Intraepithelial Neoplasia (LGPIN) and High-grade Prostate Intraepithelial Neoplasia (HGPIN) lesions were found.

The specimens were fixed in 10% buffered formaldehyde and immersed in paraffin. Hematoxylin and eosin staining of 5 \( \mu \)m specimens were done. Monoclonal antibodies to FADD and CASPASE-8 (Novocastra, UK) were used to immunohistochemical study, according to streptavidine-biotin method in dilutions 1:100 and 1:30, respectively. A semiquantitative method described protein expression. 600 cells were analyzed in each specimen under the magnification of 400×. The stained cells were counted independently of color intensity. The expression index (EI) was calculated as a percent of positive FADD or CASPASE-8 cells to total cells in specimen. In the case of FADD protein expression the EI index was termed the FADD index. The CAS index describes percent of the CASPASE-8 positive cells. The anti CASPASE-8 antibody did not discriminate proenzyme and its active form. The CAS index describes PRO-CASPASE-8 and CASPASE-8 expression. Mean values between the groups were compared using the Student t-test (p < 0.05).

RESULTS

Normal prostate epithelium was negative in both, FADD and PRO-CASPASE-8, CASPASE-8 immunohistochemistry staining. Cytoplasm of the normal secretory cells did not dye brown, similar to examined antigens in other specimens (Phot. 1). Cells observed in the pathological lesions were dark brown. PIN and prostate cancer lesions were found to strongly express FADD and CASPASE-8 proteins.

The high-grade PIN (HGPIN) lesions were characterized by the high expression of the both tested proteins when compared to low grade PIN (LGPIN). The highest expression was found in the prostate cancer lesions (Figures 1 and 2).

The FADD expression was visible in all PIN and adenocarcinomas lesions. Cytoplasm of the secretory cells was stained in the diffuse–granular manner or formed fine, dispersed grains. The color intensity was higher in cancer cells (Phot. 2) and less intensive in the PIN lesions. Expression of FADD in cancer lesions was 66,5±27,8% vs. 59,8±19,0% and 56,8±14,8% HGPIN and LGPIN, respectively (Figure 1).

Cytoplasm of the secretory cells expressed PRO-CASPASE-8, CASPASE-8 revealed homogenic pattern or can be noticed as small, dispersed grains. The color intensity was found different in both examined groups. Higher intensity was observed within the prostate cancer cells when compared to the PIN lesions (Phot. 3). The intensity of the cytoplasm staining was also different within the same specimen. The expression of PRO-CASPASE-8, CASPASE-8 in cancer lesions was 64,1±23,4% vs. 61,5±15,0% and 48,0±17,6% HGPIN and LGPIN, respectively (Figure 2).

DISCUSSION

Apoptosis is a physiological process of the cell death. Apoptosis eliminates malfunctioning cells i.e. dysplastic and neoplastic cells. Potentially dangerous and malfunctioning cells are not removed from the organism, if the mechanism responsible for programmed cell death execution fails. Cancer management and prevention strictly depends on the development in the field of programmed cell death (5). Apoptosis is regulated through many factors. These factors may act on the receptors or directly on the function of proteins of executive phase of cell death. FAS receptor (CD95, Apo-1) is a widely expressed protein in many epithelial cells including prostate epithelium (6). The changes in the expression of FAS receptor and decrease of BCL-2 mitochondrial channel protein can be connected with spread apoptosis of prostate epithelial cell in low testosterone environment. Proteins which belong to the Tumor Necrosis Factor family play a crucial role in apoptosis of prostate cancer cells after
FAS receptor presence on the prostate cancer cells makes them susceptible to apoptosis triggered after gamma radiation or chemical agents (9). No apoptosis was observed in some prostate cancer cell lines after FAS Ligand (FASL) treatment, nevertheless cells expressed receptor. The dependence between prostate cancer cells sensitivity to FASL inducible apoptosis and the degree of FAS expression was not established (8). There is a suspicion that FAS expression on the experimental cell lines and primary cultures reflects FAS expression on prostate cancer in vivo. This model can explain that incapable of apoptosis induction after FAS receptor stimulation results rather from impairment of internal proteins than chemotherapy (7).

Figure 1. Percent of the cells expressed FADD protein (FAS Associated Death Domain) within the prostate epithelium. No FADD protein was detected within the normal prostate and it was not shown. LGPIN – low grade prostate intraepithelial neoplasia, HGPIN – high grade prostate intraepithelial neoplasia, PCA – prostate cancer.

Figure 2. Percent of the cells expressed CASPASE-8 protein within the prostate epithelium. No CASPASE-8 protein was detected within the normal prostate and it was not shown. LGPIN – low grade prostate intraepithelial neoplasia, HGPIN – high grade prostate intraepithelial neoplasia, PCA – prostate cancer. LGPIN vs PCA, p < 0.05.

Phot. 1. Tissue of the normal prostate gland, stained using anti-FADD antobody (magn. 150×). No FADD expression was observed.

Phot. 2. Prostate cancer, G-3, Gleason 9, staining against FADD protein (magn. 150×). Cytoplasm of the excretory cells which expressed FADD stained in diffuse-granular or fine dispersed grains manner.

Phot. 3. Prostate cancer, G-3, Gleason 9, staining against CASPASE-8 protein (magn. 150×). Cytoplasm of the excretory cells which expressed CASPASE-8 stained homogenously or as fine dispersed grains.
from FAS receptor presence on cell membrane. Hyer et al. partially confirmed this speculation. It was observed that apoptotic answer to stimulation of FAS receptor was higher after decreasing of the mediator protein FLIP (FAS Ligand Inhibitory Protein). The P53 gene and proapoptotic BAX protein are essential to initiate FAS dependent apoptosis. DU145 and ND1 prostate cancer cell lines resistant to FAS inducted apoptosis were P53 positive and did not include proapoptotic BAX protein. PC3 and ALVA3 prostate cancer cell lines sensitive to FAS inducted apoptosis were P53 negative and expressed proapoptotic BAX protein (10, 11).

Those studies justify the research necessity in the field of intracellular factors potentially responsible for apoptosis triggering. Authors have found that huge percent (over 60%) of examined cancer cells were FADD and PRO-CASPASE-8, CASPASE-8 positive. These observations can partially explain usually prompt answer (apoptotic) of prostate cancer cells to hormonal therapy. The hormonal therapy as a first or second line management fails usually after a short period of time, probably because this apoptotic sensitive population has been removed. It could be interesting to compare cells from radical prostatectomy specimens to metastasis lesions after hormonal treatment. It should be point out that metastasis lesion as well as normal prostate gland are very difficult to obtain. One can speculate that hormonal treatment allows surviving apoptotic resistant cell population (probably less than 40% of cells in our study). Shimada has found that FAS dependent apoptosis as a basic element of chemotherapy of the prostate cancer (12). The cells expressed FAS and PRO-CASPASE-8, CASPASE-8 proteins can be sensitive for apoptotic induction. During the tumor growth cells loose their ability to apoptotic answer for FAS stimulation (13). This inability can be connected to changes in the expression of mediators and effectors of programmed cell death. In this work was found that there were cell populations „ready” to execute apoptotic programm. This population can be easily found using immunoassaying method.

Nakanishi et al. assumed that prostate cancer could be treated after a stimulation of FAS/FASL system (14). For the therapeutic purpose, stimulation expression of the protein belonging to FAS depended apoptotic pathway can be helpful.

The specimens used in this study were obtained from radical prostatectomy. This tissue was not affected with hormone treatment, but on the other side poorly differentiated tumors were selected. Authors wanted to be sure that examined cells were extremely malignant. For comparison PIN lesions were analyzed. Normal prostate tissue from young men did not express abovementioned proteins, while the huge population of the malignant cells expressed FADD and PRO-CASPASE-8, CASPASE-8. There are at least two explanations of this fact. Firstly, there is in fact no need to trigger massive apoptosis within the normal prostate epithelium, so the FAS related pathway is “silent” in almost each cell. This means, that malignant and even dysplastic cells activate apoptotic programm to counteract over proliferating effect. In this situation this program can be useful in the future for prostate cancer treatment. Protein profile indicates such cells for possible proapoptotic treatment. On the other side, cells “labeling” can give information about apoptotic resistant cell population. The second explanation is that expression of FADD and PRO-CASPASE-8, CASPASE-8 might be connected with the randomly overexpression of the tumor suppressor genes or oncogenes. Those changes can be strictly the consequence of the genomic instability. In this situation it will be difficult to choose these cells for treatment.

It was very interesting that FAS related pathway was also present in the premalignant lesions (PIN). This observation is important for chemoprevention of the prostate cancer. It was revealed that a half of the cells from PIN lesions has probably active FAS dependent pathway. That gives irrefutable proof that regulating proteins, i.e. from the BCL-2 family play a crucial role in the programmed cell death triggering within prostate dysplastic cells. Chemoprevention and cancer treatment could be more effective after complete reconstruction of FAS dependent apoptotic pathway.

It is concluded that crucial step in the prostate cancer management will be the establishing changes in FAS related protein expression. Is this a physiological response or random overexpression resulted from genetic disturbances? Do these processes coexist within the separated cell?

Clinical practice and our study suggest that there are cells, which can activate apoptotic program after receptor stimulation. The question is how to recruit and trigger apoptosis in all cells within the premalignant and cancer lesion.

CONCLUSIONS

HG-PIN and prostate cancer cells were characterized with high expression of the mediator
proteins responsible for apoptosis induction. There is a possibility that the expression of the mediator proteins responsible for apoptosis induction can be utilized as a target in prostate cancer management and even prevention.

REFERENCES


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