

THE SIGNIFICANCE OF MOLECULAR-BIOLOGICAL CHARACTERISTICS OF UPPER UROTHELIAL CARCINOMAS ASSOCIATED WITH THE BALKAN ENDEMIC NEPHROPATHY

Marina Savin, Vladimir Petronic

Institute of Urology and Nephrology, School of Medicine, University of Belgrade, Serbia

E-mail: marinas@ybc.co.yu

Summary. *The cutoff % of apoptosis (% TUNEL+ cells) for tumor grade / stage progression is twice lower in tumors generated in the regions with Balkan endemic nephropathy than outside these regions. That group of tumors more gradually invade urothelial wall than what happens in the same UUC from the general population. Apoptosis may play an important role in tumor progression, as a result of a horizontal spreading of oncogenes from an apoptotic body of one tumor cell, which is taken up by other tumor cells, as Bergsmedh et al have implied in 2001.*

Key words: *Balkan endemic nephropathy, upper urothelial carcinoma, p53, cErb-B2, apoptosis*

It is verified that upper urothelial carcinomas (UUC) associated with Balkan endemic nephropathy are frequently multifocal and bilateral, while they grow more slowly than upper urothelial carcinomas outside these areas (1). After nephrectomy for UUC (in the renal pelvis and upper part of the ureter), patients from the foci with Balkan endemic nephropathy had better survival rates than patients with similar tumors, coming outside these endemic areas, 8 against 3 years on the average (2). Both groups of tumors exhibit very similar characteristics by light microscopy assessment (1). This intriguing information have not been fully elucidated. Our investigation on a group of UUC indicates that these tumors display obvious diversity growing into grade 2 with respect to patient's settlement localization within areas with the Balkan endemic nephropathy or outside these regions ($F = 4.05$; $df1 = 1$; $df2 = 56$; $p < 0.05$). In patients from the general population, the most UUC of grade 2 rapidly invade the urothelial wall, while the same UUC in the regions with Balkan endemic nephropathy are less invasive, with the mean tumor stage 2.85 ± 1.35 and 2.13 ± 1.38 , respectively (3). This finding has coincided with rare metastases of the UUC in the endemic regions and with better patient's survival (1,2). Rapid clinical presentation of tumors from regions with the Balkan endemic nephropathy might be the additional explanation. We have analyzed possible differences in biological markers in the UUC developing in Balkan nephropathy endemic areas, by matching them up with tumor grade and stage. Biological markers of the upper urothelial carcinomas from the Balkan endemic nephropathy regions

Investigation of a group of biological markers should answer whether the UUC from endemic regions indeed have lower potential to invade, than the UUC of

the same malignancy grade outside endemic areas of Balkan nephropathy. If this notion makes sense, the classical pathologic markers of tumor malignancy (grade and stage) will not accurately display the tumor activity.

We conducted an investigation of several molecular biological markers, which evaluated tumor capacity to invade: marker of cell proliferation PCNA (PC-10), apoptosis (TUNEL test), the p53 protein status and an expression of the cErb-B2. The p53 is a checkpoint molecule in a cell cycle (in G1/S and G2 phases) enabling the repair of DNA errors and cell proliferation; otherwise it may assist in initiating apoptosis. The cErb-B2 is a kind of receptor for EGF (epidermal growth factor), a key molecule for growing of carcinoma (4). Overexpression either of p53 or cErb-B2 molecules (positive status) usually indicates their mutations.

On a group of nearly a hundred surgeries for UUC at the Clinic of Urology in Belgrade in the period 1977-2000, several tumor markers p53, PCNA and cErb-B2 were measured by immunohistochemic labeling that was carried out together with TUNEL assay on serial tumor sections fixed in 4% formaline. We have shown that these biological markers achieved some relationships, irrespectively of the place of the tumor origin, age or sex(5):

1. Proliferation marker PC10 (PCNA) is expressed on 20-25 times more cells than what is the relative number of apoptosis (TUNEL+ cells). It is the same for tumors from both endemic and outside Balkan endemic nephropathy regions.
2. The p53+ and PC10+ cells correlate with each other on the logarithmic curve.
3. The relative numbers of cErb-B2+ and TUNEL+ cells correlate.

The biological markers of tumor activity are likely to display particular dynamism in the UUC from endemic areas with Balkan endemic nephropathy (5):

1. The increases in % of TUNEL+ cells and % of PC10+ cells correspond to the exponential model in the UUC from Balkan endemic nephropathy regions. The power model better identifies that relation ($p < 0.005$) in tumors from the general population (outside endemic regions of Balkan nephropathy).

The cutoff % of apoptosis (% TUNEL+ cells) for tumor grade/stage progression is rather lower in tumors generated in the regions with Balkan endemic nephropathy. That group of tumors more gradually invade urothelial wall than what happens in the same UUC from the general population. The rates of apoptosis and proliferation are biological markers of tumor malignancy (4-9). Apoptosis may play an important role in tumor progression, as a result of a horizontal spreading of oncogenes from an apoptotic body of one tumor cell, which is taken up by other tumor cells, as Bergsmedh et al have implied in 2001 (10). It is possible that tumor p53 status is important for fusion of DNA from apoptotic body into malignant cell chromosomes. No trans-

formation of malignant cells was detected when apoptotic bodies were cultured with cells with intact p53, indicating that p53 protein may protect (tumor) cells from incorporation of activated oncogenes from apoptotic bodies (10). It would seem, not only proliferation, while apoptosis may be closely related to the rapid tumor growth and invasion, as well.

2. We found out that tumor p53 positive status (p53 protein overexpression) and % TUNEL+ cells do not correlate, although p53+ tumors have higher rates of proliferation and apoptosis. Increases in PC-10+ and 53+ cells fit in the logarithmic model for all UUC, with vigorous elevation of the representing (mean) curve for the tumors from endemic regions (not significant). It implies the influence of p53 overexpression on higher proliferation, but discards the direct significance of p53 positive status in triggering of apoptosis in tumors from the Balkan endemic regions, signifying the probable p53 mutation. Mutated p53 likewise facilitates the augmentation in chromosome aberrations by several mechanisms, one of these might be the multiplication of (onco)gene copies toward internalization of apoptotic body DNA derived from apoptosis of (the other) subclone in a tumor.

Table 1. Differences in biological markers (PC-10, TUNEL, p53, cErb-B2) in the UUC from Balkan endemic nephropathy regions and outside Balkan endemic nephropathy regions

UUC from endemic regions of Balkan nephropathy are less malignant	UUC outside endemic regions of Balkan nephropathy are more malignant
The UUC from endemic regions grow more slowly, with rare metastases and patients have better survival rates than patients suffering from the same UUC outside areas with Balkan endemic nephropathy (1-3)	The UUC outside endemic regions grow rapidly and patients have worse prognosis than those for the same UUC in the regions with Balkan endemic nephropathy (1-3)
The initial and late investigations of the UUC from and outside regions with Balkan endemic nephropathy may not reveal any difference individually in tumor grade or stage, independently of the representatives in early or advanced tumor stage (1)	The initial and late investigations of the UUC from and outside regions with Balkan endemic nephropathy may not reveal any difference individually in tumor grade or stage, independently of the representatives in early or advanced tumor stage (1)
The cutoff tumor grade for progression into stage 2 in the regions with Balkan endemic nephropathy is higher (grade 2.5) than that for the UUC outside these regions	The cutoff tumor grade for progression into stage 2 outside regions of Balkan endemic nephropathy is lower (grade 2) than that for the UUC from Balkan endemic nephropathy regions
The cut off % of PC10+cells (proliferation marker) for high grade is lower in the UUC from the Balkan endemic nephropathy regions (about 9%)	The cut off % of PC-10+cells (proliferation marker) for high grade is higher in the UUC outside Balkan endemic nephropathy regions (about 14%)
The cut off apoptosis (% of TUNEL+ cells) for high grade is lower in the UUC from the foci with Balkan endemic nephropathy, than in the UUC outside these regions (about 0.4%)	The cut off apoptosis (% of TUNEL+ cells) for high grade is greater in the UUC outside endemic regions, than for the UUC from the Balkan endemic nephropathy regions (about 0.7%)
The cut off % of apoptosis marker (TUNEL+ cells) for stage 2 is lower in the UUC from Balkan endemic nephropathy regions (about 0.2%)	The cut off value of apoptosis marker (TUNEL+ cells) for stage 2 is lower in the UUC outside the Balkan endemic nephropathy regions (about 0.6%)
The cut off % p53+ cells for high grade and /or stage 2 is similar in the UUC from Balkan endemic nephropathy regions (about >10% + cells) as in the same UUC outside Balkan endemic nephropathy regions	The cut off % p53+ cells for high grade and / or stage 2 is similar in the UUC from and outside regions with Balkan endemic nephropathy (about >10% cells)
The cut off cErb-B2+ cells for high grade is lower in the UUC from Balkan endemic nephropathy regions (>0), than in the same UUC outside these regions	The cut off cErb-B2+ cells for high grade is greater in the UUC outside Balkan endemic nephropathy regions (>1%), than in the same UUC from Balkan endemic nephropathy regions

UUC from endemic regions of Balkan nephropathy are less malignant than the same UUC outside regions of Balkan endemic nephropathy

The UUC from endemic regions may progress into high grade 3 of cell atypia with fewer portions of proliferating cells and noticeably less frequent apoptosis than UUC outside endemic regions. Accordingly, these tumors are characterized by postponed invasion (Table 1).

- The cutoff % of TUNEL+ cells for stage 2 is twice lower for the UUC from Balkan endemic nephropathy areas than for UUC outside these areas. The cutoff % of TUNEL+ cells for high grade is twice lower for the UUC in the foci of Balkan endemic nephropathy, than outside these settlements.
- In addition, the cutoff % of PCNA (PC10) + cells for tumor high grade is greater outside the regions with the Balkan endemic nephropathy.
- The cutoff % of cErb-B2+ cells for locally advanced tumor stage 2 is inversely directed in the UUC from the areas with the Balkan endemic nephropathy, in comparison to outside endemic regions (3).

In conclusion, in endemic areas with Balkan nephropathy, the increase in TUNEL+ cells and in % of cErb-B2 + cells in individual tumor fulfill the criteria for significant early prediction for progression of the

UUC. Factor analysis indicates that these two markers, TUNEL and cErb-B2, better represent the disparity between UUC from endemic and outside endemic regions, than classical tumor grade or stage, or p53 status and proliferation marker PC-10.

Obvious dissimilarity in cutoff point apoptosis for expansion of the UUC from Balkan endemic nephropathy regions (lower apoptosis rate) in comparison to that of the UUC from the general population (greater apoptosis rate) we have represented in this review, may help provide an explanation for slow invasion of the UUC associated with the Balkan endemic nephropathy. Lateral transfer of DNA from apoptotic bodies between cells may result in accumulation of genetic changes that are necessary for tumor promotion (10).

It is very important to evaluate the contribution of these biological markers to (overall) survival rate of the patients with the UUC from areas of Balkan endemic nephropathy, in which UUC occurrence is a hundred times and more frequent than it is in the general population. Similar manifestation and clinical course of the UUC associated with endemic nephropathy and with analgesic nephropathy are in favor of toxic etiology of both UUC (1,11). Parallel investigations of biological markers in these tumors should answer whether urothelial malignancies in analgesic abusers and from the regions with the Balkan endemic nephropathy have particular (similar) pattern of evolution and whether they share the same biological markers of tumor progression.

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