Apoptosis and p53 status of the upper urothelial carcinomas from Balkan endemic nephropathy regions

V. Petronic´ and M. Savin

Institute of Urology and Nephrology, Belgrade, Yugoslavia

Introduction

The upper urothelial carcinomas (UUC) associated with endemic nephropathy and analgesic nephropathy are considerably similar in many characteristics. These similarities diversify them from the same carcinomas in the general population [1]. Bilateral occurrence (simultaneous or successive) of the UUC is observed in about 8% cases of analgesic nephropathy and 10–14% in endemic Balkan nephropathy. Multiple occurrence is seen in up to one-third of cases, with every 15th patient developing cancer in the urinary bladder. By regular examinations for UUC during several years on haemodialysis, the tumour prevalence increased to 16 against 1 in endemic or analgesic nephropathy and remained 1 against 50 in patients with other kidney diseases. The maximum incidence of UUC in endemic regions is in the sixth to seventh decades of life. Those UUC slowly expand with rare metastases outside endemic regions; the median time of the UUC growth following clinical presentation is two times shorter. An epidemiological survey of the group of nephrectomized patients at the Clinic of Urology of Belgrade revealed a better survival of UUC patients from endemic regions, 8 against 3 years on average [2]. The impression of a slow evolutionary process contrasts with the presence of advanced tumour malignancy grade II and III. Routine histologic examination did not reveal any significant differences in characteristics of the UUC in relation to the settlement in large groups of patients during several decades.

Aetiology

In the regions where Balkan nephropathy is endemic, the maximum incidence of UUC is a 100 times greater. Clustering of both diseases in several members of a family questions the role of hereditary factors inducing the development of endemic nephropathy and associated UUC. Our epidemiological survey could only support the fact that two entities occur simultaneously in the same population, rather than in the same individuals. Frequent chromosomal aberrations on 3q were specified for UUC from endemic regions [3]. In the last few years a toxic aetiology of endemic nephropathy has been implicated. Bach et al. [4] indicate that ochratoxin A is a nephrotoxin that may be involved in the pathogenesis of endemic nephropathy and UUC. They developed a rat model where exposure of the urothelium to ochratoxin A (given in drinking water) and acute papillary necrosis induce UUC [4]. Ochratoxin A adducts were present in the DNA in urine and tissue samples of the patients with UUC from endemic regions [5]. All these data support the thesis that the biological features of UUC in endemic and outside endemic regions are different.

Apoptosis index—a biomarker of UUC in endemic regions

Carcinomas have been shown to evolve as a consequence of genetic alterations and chromosomal instability. Some gene mutations enhance cell proliferation, so that the transformed cells become prone to subsequent genetic mutation. Some of those mutations affect the stability of the entire genome, which will increase the overall mutation rate. However, apoptosis also occurs in a cancer. An accumulation of genetic alterations augments the susceptibility of a malignant cell to apoptosis as a somewhat genetically dysregulated ‘programmed cell death’. Apoptosis in a cancer may not prevent tumour progression. Theoretically, apoptosis is involved in the selective growth of a subclone and plays a role in the tumour development. Besides, other factors in a tumour could induce apoptosis of a malignant cell, such as ischaemia, a toxic effect of a drug or radiation. In respect of the possible toxic aetiology of endemic nephropathy and associated tumours, apoptosis and proliferation in these tumours might have different kinetics than in other UUC. Multifocality of urothelial carcinomas has
been attributed to seeding of exfoliated tumour cells and or a general sensitivity of the entire urothelium to carcinogenic stimuli. A clonal origin of the majority of multifocal superficial urothelial cancers of a low-grade has been suspected. They are genetically stable, despite their frequent recurrence. Differences between tumour and normal epithelium were observed in the frequency of chromosomal aberrations. Recent data suggest a general genetic instability as a reason for multifocality in the entire transitional epithelium. Petersen et al. support the view that phenacetin causes urothelial carcinomas through chronic tissue damage (field theory) rather than by promutagenic DNA lesions [6].

Applying different methods on tissue samples, we confirmed the presence of apoptosis in the UUC from endemic regions [7]. Selective binding of anti-ssDNA antibodies to nuclei in early apoptosis reflects instability of DNA to denaturation by heating. Added MgCl₂ prevents DNA denaturation in non-apoptotic cells. The late apoptosis with nuclear degradation by inter-nucleosomal fragmentation of DNA is detected in a TUNEL assay following low protease K activity. Masuda et al. indicated fast progression of the UUC with IxTUNEL > 0.22 [8]. Our investigation on tumour tissue samples in a group of 40 advanced cases with UUC from endemic regions shows that IxTUNEL > 0.2 predicts tumour progression into high grade or kidney invasion, respectively (OR = 5.53, P = 0.0024; OR = 3.59, P = 0.0068). Outside endemic regions, UUC in grade III had similar occurrence of apoptosis as tumours in grade II. The receiving operating curves (ROC) of specificity and sensitivity of three biological markers displayed obviously distinctive patterns for the UUC from endemic regions. The cut off value of IxTUNEL for invasive tumours in endemic regions was twice less (0.37) than that for the same tumours outside endemic regions (0.63). Fifty per cent of either number of tumours from endemic (20/20) or outside endemic regions (17/30) exposed high IxTUNEL. Proliferation IxPCNA in excess of 10.12 in endemic and 14.5 outside endemic regions indicate tumours in either high grade or with the p53+ status by immunohistochemistry.

Results argue for the rare presence of apoptosis in low-grade UUC in endemic regions. If the putative toxins were operating against a background of genetic instability and carcinogenesis; they would not contribute to frequent apoptosis. Higher degrees of apoptosis are present in UUC with extreme cell atypia (grade 3). In addition, in endemic regions proliferation does not dramatically increase with UUC progression to high grade, as others and we have observed for UUC in the general population. Based on the significant decrease in the proliferation to apoptosis ratio between the UUC grade II and III that is only observed in endemic regions, we suppose a peculiar cell-cycle deregulation in these tumours in comparison to other UUC. It could reflect a slower evolution of tumours from endemic regions. The UUC from endemic regions show an exponential increase (\( y = 0.1279 \times \text{ex}^{0.053}; P < 0.05 \)) of the TUNEL+ cells depending on the proliferation IxPCNA, which is on average 20–25 times higher. The same relation in the UUC outside endemic regions fits to the power model (\( y = 0.0443 \times \text{ex}^{0.8346}; P = 0.01 \)). In UUC outside endemic regions, proliferation of cells with high-grade atypia dramatically increases. It would seem that the genetic aberrations are different in tumours from endemic and outside endemic regions.

The p53 status of the UUC in endemic regions

The UUC from endemic regions, overexpressing p53 protein did not differ in apoptosis from the same tumours outside endemic regions; the p53 status and apoptosis do not correlate in tumours of both groups [9]. Accumulated wtp53 could transactivate the pro-apoptotic gene Bax in which case it triggers downstream caspases, the major effectors of apoptosis. Mutated p53 gene loses its suppressor function in maintaining genome stability. Some mutations in the specific sequences of p53 would effect a new p53 transactivation, what wtp53, as a tumour-suppressor protein could not induce (‘gain of function’). The PCNA has been subjected to wild-type p53 cell-cycle negative control, by p53 transactivation of cdk/PCNA inhibitor p2I. Mutated p53, as an oncoprotein could directly transactivate several oncogenes, among those is the same DNA polymerase co-enzyme PCNA (positive control). Mutated p53 protein escapes degradation and prolongs its half-life to 2 h, enabling immunohistochemical detection and the majority with the p53+ tumours represents mutated p53 protein, reflecting advanced stage of carcinogenesis. We discovered p53+ (>20%+ nuclei) applying MoAb-DO7 that recognized amino acids 20–25 epitopes on wild-type and mutated p53 protein. The p53+ tumours account for 37% UUC from endemic and 38% UUC outside endemic regions. T1 carcinomas of the urinary bladder have similar frequency of the p53 positivity [10]. In our series, 50% out of 40 UUC with >10% p53+ cells were developing outside endemic regions and had significantly higher proliferation (IxPCNA: \( 27 \pm 17 \) to \( 16 \pm 11\%; F = 6.024; P = 0.019 \)). Increase in IxPCNA and the p53+ status fit to the logarithmic model for all UUC, although the curve for tumours from endemic regions rapidly increases (Figure 1).

Late increase in apoptosis in tumours from endemic regions

An increase in apoptosis does not necessarily reflect an enhanced malignant potential of a tumour. Chronic ischaemia may facilitate triggering of apoptosis in a tumour. Endemic nephropathy is a slowly progressive disease with extensive tubular atrophy and interstitial fibrosis. It may result in the smallest kidneys of only a few cm in length. In endemic nephropathy, the associated UUC slowly grow in conditions of surrounding ischaemia. Renal insufficiency more frequently accompanies these tumours [1]. Apoptosis may be caused by these two conditions: the progression of tumour...
malignancy on the one hand, the oxidative stress in chronic ischaemia on the other. Vice versa, increased apoptosis may affect tumour remodelling and growth. However, a substantial number of patients with UUC in endemic region do not develop endemic nephropathy.

**Conclusion**

The determination of biomarkers in the UUC from endemic regions may contribute to a better understanding of these tumours. Apoptosis displays different kinetics in tumours from endemic regions and represents a useful marker of early prediction of tumour progression. Further molecular investigation of the UUC associated endemic and analgesic nephropathy is recommended.

**References**

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