Letter to the Editor

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Downregulation of Checkpoint Protein Kinase 2 in the Urothelium of Healthy Male Tobacco Smokers

Johannes Breyer[a] Stefan Denzinger[a] Arndt Hartmann[b] Wolfgang Otto[a]

[a]Department of Urology, Medical Center St. Josef, Regensburg University Medical Center, Regensburg, and
[b]Institute of Pathology, Friedrich-Alexander University of Erlangen-Nuremberg, Erlangen, Germany

Key Words
Bladder · Bladder carcinoma · Immunohistochemistry · Transurethral resection · Etiology · Risk factor · CHEK2

Abstract
With this letter to the editor we present for the first time a study on CHEK2 expression in normal urothelium of healthy male smokers, former smokers and non-smokers. We could show a statistically significant downregulation of this DNA repair gene in current smokers compared to non-smokers, suggesting that smoking downregulates CHEK2 in normal urothelium, probably associated with an early step in carcinogenesis of urothelial bladder carcinoma.

Urothelial bladder carcinoma (UBC) belongs to the 5 most frequent tumors occurring worldwide. In a recent meta-analysis, Burger et al. [1] confirmed that about 50% of all bladder tumors are derived from tobacco smoking. Samanic et al. [2] analyzed a collective of 2,500 patients in Spain, where UBC incidence among men is the highest in the whole EU [1, 2]. Current smokers had a 7.4-fold risk to develop UBC, while former smokers still had a nearly 4 times higher UBC incidence than non-smokers. While overall there was no statistically significant decrease of UBC risk in former smokers, quitting the habit of smoking blond tobacco for more than 10 years led to a statistically significant lower tumor incidence compared to current smokers [2].

The difference between blond and black tobacco lies in black tobacco's much higher concentration of N-nitrosamine and 2-naphthylamine. However, it is unknown how these substances bring about carcinogenesis in detail. It would be most valuable to study the consequences of tobacco smoking on histopathologically normal urothelium to recognize very early molecular alterations. In the present preliminary study, we performed an immunohistochemical analysis of healthy male urothelium of current, former and non-smokers without histopathological signs of urothelial changes for the first time in vivo and identified expression of checkpoint protein kinase 2 (CHEK2). To repair cellular damages by carcinogens, protein kinases like CHEK2 are activated to introduce repair mechanisms and stop cell cycle until the damage is repaired. Downregulation of CHEK2 has been proven in various neoplasms, for example, lung cancer, to be associated with worse outcome [3]. Germline mutations in CHEK2 are reported to be associated with an increased risk of bladder cancer [4].

After patients' informed consent following a positive vote of the local Ethics Committee at Regensburg University, macroscopic unsuspicious urothelial specimens of the bladder were taken from 92 male patients during transurethral resection of the prostate. Samples were assessed by an experienced uropathologist (A.H.) and revealed normal urothelium without histomorphological changes. A tissue micro array was constructed and stained with CHEK2 protein kinase antibody (mouse mAB 3440, dilution 1:3,200). As in previous immunohistochemical analyses on CHEK2, we compared negative and weak to moderate and strong staining (fig. 1a, b).

Of 92 patients (median age 70, IC 64–76 years), 45% (n = 41) did never smoke, 43% (n = 40) stopped smoking at least one year ago and 12% (n = 11) were current tobacco smokers. Moderate and strong staining of CHEK2 was shown in 44% of non-smokers but only in 9% of current smokers with a statistically significant difference (p =

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PD Dr. med. Wolfgang Otto, Assistant Prof.
Department of Urology
University of Regensburg
Landshuterstrasse 65, DE–93053 Regensburg (Germany)
E-Mail wolfgang.otto@ukr.de

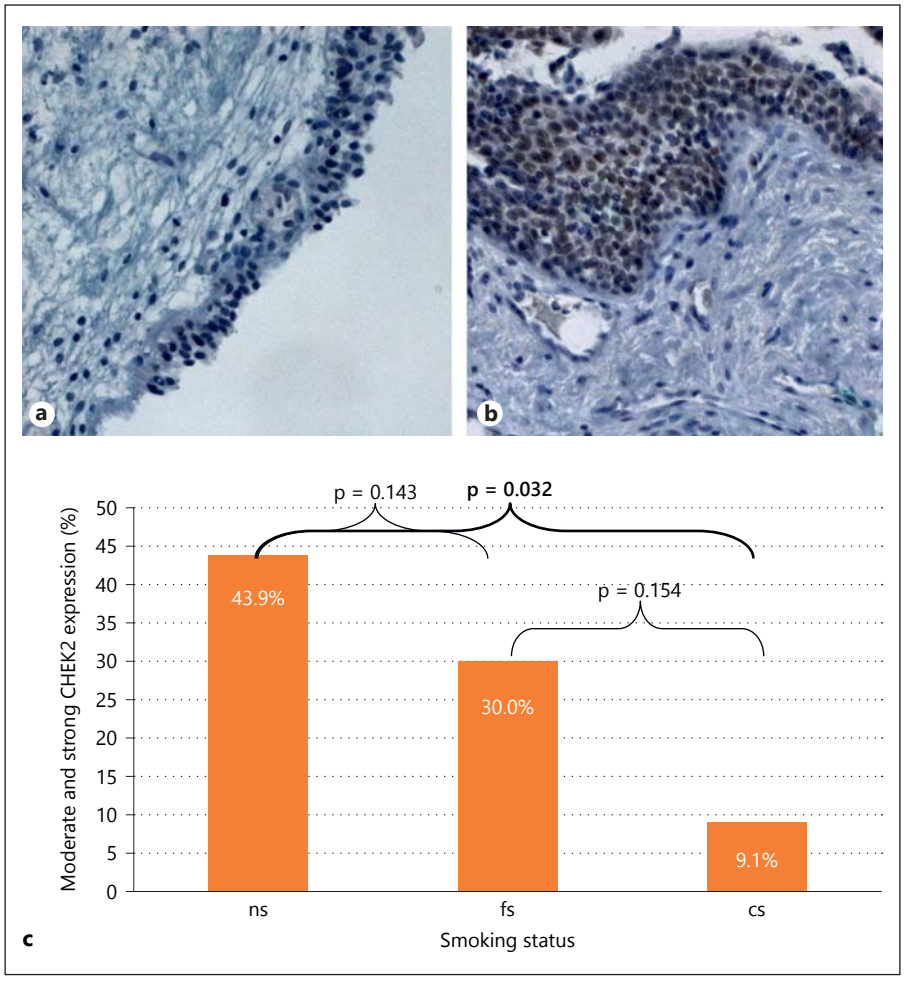

Fig. 1. a–c Examples of negative (**a**) and strong positive expression (**b**) of CHEK2 in urothelial cells of healthy male patients (40-fold magnification). **c** Percentage of moderate and strong expression of CHEK2 in urothelial specimens of non-smokers (ns), former smokers (fs) and current smokers (cs). Statistically significant p values in bold.

0.032), while former smokers regained a higher rate of CHEK2 expression (30%; fig. 1c). This suggests that tobacco smoking downregulates CHEK2. This might be a first step in the development of urothelial neoplasms. There was no statistically significant difference in the expression of CHEK2 between former smokers and non-smokers suggesting that effects of tobacco smoking on CHEK2 expression are reversible, when smoking is stopped. Further studies on the effects of tobacco on human urothelium are needed to reveal the very first steps of tobacco-derived carcinogenesis in the urothelium.

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