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Role of HPV in Urothelial Carcinogenesis: Current State of the Problem

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1. Introduction

Human papillomaviruses (HPV) of the so-called high risk types (HR-HPV) cause cervical cancer (CC). Carcinomas in other organs such as vagina, vulva, penis, oropharynx and rectum are known to be aetiologically heterogeneous with respect to HPV (zur Hauzen, 2000, 2008; Gillison & Shah, 2003; International Agency for Research on Cancer [IARC], 2008). HPV-positive cancer in those organs including cervix uteri differs from HPV-negative one in molecular-genetic profile, morphology as well as in clinical peculiarities (Morrison et al., 2001; Gillison & Shah, 2003).

Carcinogenicity of the HR-HPV is determined by two viral genes, *E6* and *E7*. Their expression is recognized as a necessary condition for conversion of virus-infected cell from normal to malignant state. Viral oncoproteins E6 and E7 can interact with various cellular proteins and thus preclude their normal functioning. Among numerous activities of viral oncoproteins the following two are usually regarded as principal ones. E7 is capable of binding to retinoblastoma protein pRb, and E6 can interact with p53. Therefore both above mentioned tumor suppressors become inactivated and then degraded. Cellular functions such as proliferation, apoptosis, DNA repair etc., controlled by pRb and p53, become disturbed (zur Hauzen, 2000, 2008; IARC, 2008).

Since CC is a frequent female malignancy many research groups were occupied in search of early diagnostic markers for this cancer type. Experience thus obtained extends usually to HPV-associated carcinomas of other organs after necessary validation. Attempts to detect HR-HPV DNA by PCR did not leads in those studies to designing of a reliable diagnostic test because cancer *in situ* and invasive CC developed in a small proportion of women with HR-HPV-positive dysplasia (zur Hauzen, 2000). So specificity of the given approach turned out to be low despite the known very high PCR sensitivity. Current attempts to improve early diagnostics of CC and some other HPV-associated cancers are mostly focused on search of genes in virus-infected host cell whose expression becomes unconvertably altered under the influence of viral oncoproteins (Santin et al., 2005).

One of these genes is *INK4a* encoding $p16^{INK4a}$ protein, an inhibitor of cyclin D-dependent kinases Cdk 4/6 (Serrano et al., 1993). *INK4a* transcription in displastic and cancer cells becomes much more active in comparison with its level in normal epithelium being triggered by HR-HPV oncoprotein E7; the content of $p16^{INK4a}$ in a cell increases correspondingly (Li et al., 1994; Khleif et al., 1996; Sano et al., 1998; Kaneko et al., 1999; Klaes

et al., 2001). This phenomenon formed experimental grounds for the immunohistochemical test which is currently widely applied in early CC diagnostics (Klaes et al., 2001, 2002; Milde-Langosch et al., 2001; Volgareva et al., 2002, 2004, 2006). This test is becoming popular in diagnostics of HR-HPV-associated carcinomas of other localizations (Begum et al., 2007; Kim et al., 2007).

Bladder cancer (BC) takes 7-th place in the global cancer incidence making up ~ 2-5% of all neoplasms. BC is 2.5-6 times more frequent in men than in women: 260000 new BC cases are registered annually among men and only 76000 among women. Bladder tumors are rare in people under 35 years old; however BC has become younger recently (Parkin et al., 2003). It seems reasonable to mention in this connection the data of Scandinavian investigators (Litlekalsoy et al., 2007) concerning dynamics of the BC molecular markers. They reported that significant shift in the BC molecular profile occurred during 70 years. This shift possibly reflects some alterations in the set of BC causative factors which might have taken place during these years.

In Russia BC makes up ~ 3 % of all malignant tumors; the trend has been registered for the steady elevation of new cases number (Chissov et al., 2010). Mortality among male BC patients in Russia is higher (> 7 in many regions) than the highest indices for countries from the WHO mortality list; as to female BC patiens, mortality figures do not differ in this group from those in other European countries (Zaridze, 2009).

BC development is a multistage process with unpredictable course. Several risk factors for BC are known (Zaridze et al., 1992; Dinney et al., 2004). Among these factors are: geographic region (BC morbidity may vary worldwide up to tenfold); professional occupation (there are about 40 professions at high risk); smoking; nutritional habits and drinking water quality; use of certain medicines; parasitic diseases caused by some Trematoda (Schistosomas). Possible significance of some other factors is still under discussion including irradiation, hereditary predisposition, some other.

Association of some biological agents with BC development might be suggested from results of one study in ~ 6000 patients cohort (Adami et al., 2003). Various organs had been transplanted to those patients with consequent immunosuppressor treatment. BC incidence in this group turned out to be 2-4 times higher as compared with corresponding index for the population as a whole. Carcinomas with proven causative role of HPV occurred in this group of patients even more frequently. Thus prevalence of vulvar and vaginal cancer was 20 times higher than expected one, and that for rectal and oropharyngeal cancer – 10 and 5 times higher, respectively.

The problem of HPV involvement in urinary bladder carcinogenesis is not novel. Historically one of the first indications to the possible linkage between these viruses and BC was the fact that secondary BC occurrence in women with primary CC was significantly higher (five to six-fold) than its occurrence in general population (Bailar, 1963; Newell et al., 1974, 1975). The interpretation of that data in favor of real HPV involvement in urothelial carcinogenesis became possible later after the discovery of HR-HPV carcinogenicity for cervical epithelium by H. zur Hausen and co-authors (Durst et al., 1983, zur Hauzen, 2000, 2008). However definitive commentary on those results as proving HPV carcinogenicity for urinary bladder is still difficult due to the known fact that both CC and BC are more frequent among smoking women.

One more evidence in favour of HPV involvement into BC development was obtained in observations carried out on immunodeficient patients with benign or malignant bladder

neoplasms where HPV DNA was found (Del Mistro et al., 1988; Kitamura et al, 1988; Querci della Rovere et al., 1988; Maloney et al., 1994).
The International Expert Group on HPV selected over twenty studies dedicated to HPV role in BC which had been published in 1991-2001 worldwide. The authors had detected HPV DNA in BC specimens by PCR, *in situ* hybridization and/or Southern blot hybridization. Percentage of HPV-positive cases in these communications varied from 0 up to 82.6 %. Therefore the experts included BC into the category of cancers for which aetiological role of HPV remains unclear – "inadequate evidence" (IARC, 2008).

2. The recent data warn against HPV underestimation as a risk factor in urinary bladder carcinogenesis

Several researchers have published recently some data proving topicality of the HPV problem in BC aetiology (Barghi et al., 2005; Yang et al., 2005; Helal Tel et al., 2006; Moonen et al., 2007; Badawi et al., 2008).
Thus ~ 36 % of transitional-cell BC specimens from Iran (21 out of 59 studied) harboured HPV DNA (Barghi et al., 2005). HPV 18 predominated over other types of the viruses (it was found in 17 patients out of 21, - 81 %); - viruses of the given type are second most frequent causative agents for CC (the first place belongs to HPV16). Urinary bladder tissues from 20 non-oncological patients were taken for control in this study and HPV18 DNA was detected in 1 patient with heavy cystitis. Possibility of precancerous alterations in the latter case could not be ruled out. The authors concluded that HPV may play role of a causative BC factor.
Similar was the opinion by the researchers from the Netherlands who carried out study of BC specimens from 107 patients and found DNA of various HPV types in ~ 15 %; HR-HPV DNA was detected in ~ 8 % (Moonen et al., 2007). Percentage of HR-HPV DNA-positive specimens increased with progression of clinical stage of BC (Ta, T1 and T2-T4), making 0, 12.5 and 18.2 % respectively
Group of investigators from Egypt and USA presented data on HPV-positivity of 27 Schistosoma-associated BC cases (Yang et al., 2005). All of them harboured HPV16. Highly sensitive variety of PCR was used in the study. The results reported by another group from Egypt (Helal Tel et al., 2006) differ dramatically from the data of H. Yang et al. These authors found HPV 16/18 DNA in a single Schistosoma-associated BC specimen (squamous cancer *in situ*) out of 64 studied. Much lower sensitivity of *in situ* hybridization used in the last study for HPV DNA detection in comparison with the method used by H. Yang et al. may be responsible for such a sharp data discrepancy. The total sum of BC specimens examined by A. Helal Tel et al. was 114 including 67 transitional-cell, 32 squamous and 15 other. The above mentioned case was the only HPV-positive BC in this study. The results obtained enabled the authors to conclude that HPV do not play any significant role in pathogenesis of urinary bladder in Egypt.
The data reported by these two research groups, H. Yang et al. and A. Helal Tel et al. and mutually exclusive inferences made by the investigators warn against underestimation of HPV as a risk factor in BC genesis. Essential in this connection is the fact that results reported by H. Yang et al. were confirmed recently by another research group from Egypt (Badawi et al., 2008). The authors using PCR detected HR-HPV DNA (belonging to types 16,18 and 52) more frequently in BC specimens than in urothelial biopsies from cystitis patients. The PCR data were compared with the data on antibody to HPV16 protein L1

detection in blood serum of the HPV16-positive BC patients; perfect coincidence of these results took place. The association was observed in this study between HPV-positivity of BC and its propensity for relapse. The authors concluded that HPV participates in BC genesis in combination with other risk factors, including Schistosomas which were commonly found in the group of patients examined. The authors recommend detection of antibodies to HPV L1 to optimize the treatment of BC patients and their further follow-up .

3. Aspects of the problem to be addressed

Thus a glimpse into the problem of HPV role in BC gives idea of its complexity. Therefore we rise the following questions:

1. What reasons may be the for conflicting data communicated by different research groups? Are there any ways to optimize the methodology of the study and get uniform data?
2. What is the incidence of HPV-positivity among urothelial dysplasia and BC specimens obtained from Russian patients keeping in mind ethno-geographic BC heterogeneity?
3. Have there been any attempts to investigate the role of papillomaviruses in urothelial carcinogenesis in experimental models?
4. What benefits may it bring to practical oncourology provided that a certain role of HPV in BC is accepted by medical community?

4. What reasons may be for conflicting data communicated by different research groups? Are there any ways to optimize the methodology of the study and get uniform data?

The authors usually explain the conflicting data character by different research groups by either of the following factors:

1. objective ethno-geographic heterogeneity of BC and
2. technical peculiarities of studies.

Concerning the first factor, a relationship seems to be evident between the state of the excretory organ lining, on the one hand, and environmental factors such as drinking water quality, regional and ethnic specificity of food, endemic urinary bladder parasitic diseases, etc., on the other hand. Each of these factors may influence the HPV-BC association rate. This statement could be verified by comparison of HPV-positivity in BC from different regions worldwide done by the same research group with unified technical approaches. Such studies have never been carried out as far as we know .

The second group of factors includes small numbers of specimens tested in some works; application of a single test for viral DNA detection (most commonly PCR or *in situ* hybridization, wherein both techniques have benefits and limitations); detection of only one or two HPV types, usually HPV16 and HPV18, which are most frequent in CC, while other HPV types might be involved in carcinogenesis in urinary bladder. The data by C. De Gaetani et al. prove the latter point (De Gaetani et al., 1999). Using *in situ* hybridization with probe to 31/33/35 viral types the authors detected HPV DNA positivity in 60 % of BC specimens, while the index turned out to be 24 % with probe to the types 16/18 .

In addition, a predominant majority of groups which publish data on high incidence of HR-HPV DNA in BC made no attempts to confirm viral genome expression and in particular *E6/E7* expression.

Contamination should be mentioned also besides the above-listed factors. It may be either laboratory (admixing of products of viral genome amplification to the samples under study) or patient related. The former is a well-known source of false-positivity of PCR data. Possibility of the latter is to be kept in mind in studies of BC specimens particularly. If any adjacent organ (vulva, penis, urethra) is HPV-infected, casual bladder contamination with HPV-harbouring cell(s) might occur through blood during surgical operation or by endoscope during cystoscopy. False HPV-positivity data may occur both in PCR done to screen materials for viral DNA presence and in reverse-transcription PCR (RT-PCR) study of viral genome expression as well.

Thereby complex approach seems to be reasonable to study possible HPV role in urothelial carcinogenesis. Techniques are reasonable which enable to detect DNA not only of HPV16 and HPV18 but of other types of viruses as well. To check up whether viral oncogenes *E6* and *E7* are expressed in DNA-HPV-positive specimens methods seem to be appropriate of both viral mRNA *E6/E7* detection (by RT-PCR) and viral oncoproteins E6 and E7 revelation (by immunohistochemistry). In female BC cases it may be reasonable to examine patient's cervical epithelium for HPV infection.

5. What is the incidence of HPV-positivity among urothelial dysplasia and BC specimens obtained from Russian patients?

The given section presents data of two independent studies of Russian patients with urinary bladder oncological conditions including results of our own complex approach to HPV detection in BC specimens.

5.1 Attempts to determine occurrence of HPV-positivity in bladder urothelium

DNA of HPV16 and HPV18 was found in ~ 50 % of urinary bladder dysplasia and carcinoma in situ specimens by in situ hybridization; an attempt to detect HPV of other types (6, 11, 31, 33 and 51) gave negative results (Frank et al., 2002).

We have screened 130 transitional BC specimens (1-3 grade) obtained by transurethral resections for HPV DNA using several PCR versions with primers to *L1, E6* and *E7* genes of the viral genome (Volgareva et al., 2007, 2008, 2009; Trofimova et al., 2009). Our tests included application of literary primers My09/11 and GP5-GP6 to *L1* enabling one to detect HPV of various types; these primer sets are commonly used in similar studies (Resnik et al., 1990; van den Brule et al., 1990). HPV16 genetic material was found in ~ 40% of the specimens tested, DNA of other HPV types was not found. Viral genome expression was confirmed at the level of mRNA by RT-PCR in some of the specimens (Volgareva et al., 2009; Trofimova et al., 2009). Viral oncoprotein E7 was spotted by immunohistochemistry in ~30% of DNA HPV16-positive cases (Cheng et al., 2009; Volgareva et al., 2009 a,b). BC specimens stained positively with polyclonal anti-E7 HPV16 serum (done by Fiedler et al., 2004) turned out to be positive also when stained by monoclonal antibodies to HPV16 E6 and E7 from Neodiagnostic (Cheng et al., 2009). Five examples of BC specimens' screening for HPV are presented in Table 1 and Figure 1.

The fact that HPV16 oncoprotein E7 is detected in ~30% of BC specimens means that HPV16 plays some role in urothelial carcinogenesis in Russian patients. However in case of urothelial malignization some deviations there seem to exist from the known role of these viruses in cervical carcinogenesis. Signs testifying to the truth of the given assumption are as follows.

Case No	DNA*	RNA**	Protein E7*** (type of staining)
1	-	not studied	-
2	+	+	+ (diffuse)
3	+	+	+ (diffuse)
4	+	-	+ (focal)
5	+	-	+ (focal)

* HPV DNA was detected by PCR, viral typing carried out either by PCR with type-specific primers or by restriction fragment length polymorphism test (Astori et al.,1997). Specimens 2-5 appeared to harbour DNA of HPV16.
** reverse-transcription PCR was carried out with primers to *E6/E7* HPV16.
*** immunohistochemical staining was performed with polyclonal serum to HPV16 oncoprotein E7 (Fiedler et al., 2004). The type of staining was either diffuse (over 25% of stained cells in a cancer tissue) or focal (less than 25% of stained cancer cells).

Table 1. Data of the complex approach to HPV detection in five BC specimens. Case 1: Transitional BC relapse, focuses of squamous metaplasia, 3-d grade, muscle-invasive. Case 2: Transitional BC, 3-d grade, muscle-invasive. Case 3: Transitional BC, 2-d grade, submucosal invasion, no muscle cells on the slide. Case 4: Transitional BC, focuses of squamous metaplasia, 2-3-d grade, growth within mucous layer, no invasion into muscle. Case 5. Transitional BC, 3-d grade, submucosal invasion, no muscle cells on the slide.

Firstly, along with BC specimens expressing viral oncoprotein E7 in a predominant majority of cancer cells throughout cancer tissue (as was usually the case with CC in our previous studies, - the so-called "diffuse staining"; - Volgareva et al., 2006) we observed some BC cases in which E7 was registered only in certain groups of cancer cells or in separate cells, - the so-called "focal staining" (Table 1, cases 4 and 5; Fig. 1d,e). It should be underlined in this connection that we confirmed HPV16 genome expression at the level of mRNA by RT-PCR for some of such BC specimens. However it was not in every BC specimen studied that the results of RT-PCR and immunohistochemistry coincided: cases 4 and 5 in Table 1 serve as examples of the lack of the data homogeneity. Focal character of HPV16 genome expression registered immunohistochemically may perhaps be responsible for this discrepancy: HPV16-harbouring cells detected by staining in a certain section of a BC specimen might not occur in another section of the same specimen from which mRNA was obtained. It is also important that in all such specimens HPV16 E7-expressing cells were found in the internal layers of cancer tissue but not at its brims (Fig. 1d,e). This observation enables one to rule out the above-mentioned possibility of the urinary bladder intrapatient contamination with cells of some adjacent HPV-infected organ. Focal HPV16 E7 expression in some BC specimens in our study is in a good agreement with the data by C. De Gaetani et al. on HPV DNA detection in BC by *in situ* hybridization (De Gaetani et al., 1999). These investigators had at their disposal several biopsy samples for each of ten patients under study. It was from only one out of ten patients that the test results were permanently positive in all biopsies, while in the rest nine cases only a quota of samples was DNA HPV-positive.
Secondly, in some cases of focal E7 expression BC cells contain this viral oncoprotein only in a cytoplasm (Fig 1e). Its ability to get bound to the nuclear pRb remains under question in such cases.

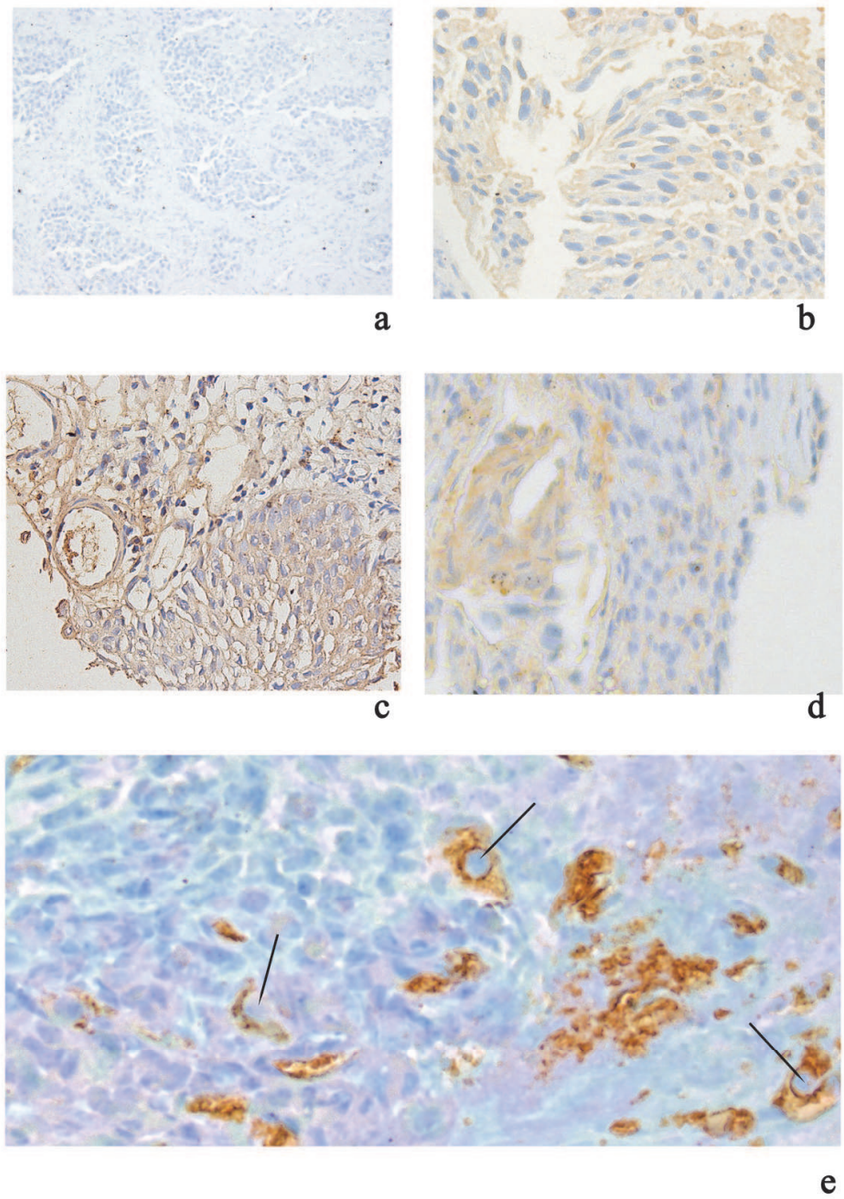

Fig. 1. Results of the HPV16 E7 immunohistochemical detection in BC specimens. Specimens' numbers match to those in Table 1. a Negative reaction with E7-specific serum in specimen N1. b, c Diffuse staining of specimens NN 2 and 3, respectively. d, e Focal staining of specimens NN 4 and 5, respectively. Uncoloured nuclei of three cells expressing E7 in a cytoplasm are indicated with arrows in "e".

Thirdly, the results of our repeated examination of the female patient with relapsing BC turned out to be quite unexpected. In her original tumor removed surgically in 2004 we detected HPV16 DNA, *E7* mRNA as well as protein E7 (the latter spotted independently in two laboratories with different antibodies) (Volgareva et al., 2009 b; Cheng et al., 2009). The patient is a hard smoker. For more than 20 years she had worked at a chemical factory and had been exposed with solvents and aniline dyes. Three BC relapses took place in 2005-2008.

During this period the patient undervent surgery, chemotherapy and BCG treatment. At the next relapse in 2009 we performed repeated study. Neither HPV DNA nor protein E7 were found in BC cells. Colposcopy study was also performed and HPV DNA tested in cervical cells by PCR; the results of both analyses proved absence of HPV in cervical epithelium of the patient (Volgareva et al., 2010a). Could there occur a total clearance from virus-harbouring cells due to surgical and other treatments in this patient? Further observations on similar cases are desirable to answer in the affirmative.

5.2. Study of *INK4a* expression in DNA HPV16-positive bladder cancer specimens

To verify the fact of HPV16 genome expression in 50 DNA HPV16-positive BC specimens we studied cellular *INK4a* expression at the levels of mRNA (Fig. 2) and respective protein $p16^{INK4a}$ (Fig. 3) (Volgareva et al., 2010b). The above mentioned phenomenon of the *INK4a* overexpression indicating to HR-HPV E7 activity in cervical cells served as a rationale. In 12 BC specimens under study the HPV16 *E7* expression had been detected at the mRNA and/or protein level. Five conditionally normal urothelial specimens obtained from the same BC patients were studied as well. In some BC cases associated with HPV16 DNA we detected *INK4a* overexpression at the both levels (Fig. 2, patients A,B and E; Fig. 3c).

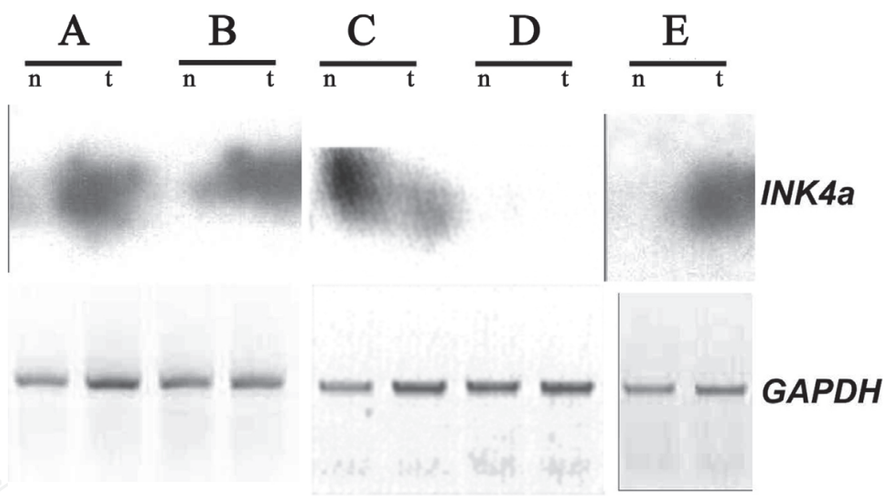

Fig. 2. Analysis of expression of *INK4a* by RT-PCR in BC specimens obtained from five patients (A, B, C, D and E); t - urinary bladder carcinoma, n – morphologically normal tissue adjacent to tumour.
The top-panel electrophoregram developed after Southern blot hybridization with the *INK4a*-specific radio-active probe according to Nguyen and co-authors (Nguyen et al., 2000).
The bottom panel: results with *GAPDH*-specific primers as a control for stability and concentration of RNA; amplification products visualized by staining with ethidium bromide.

Incidence of $p16^{INK4a}$- overexpressing BC specimens was ~ 10% (Fig. 3c), however as opposed to CC in BC it did not correlate with HPV16 E7 expression in any case (Figures 2c and 3d present lack of such correlation for one and the same BC specimen).

We don't regard this result as evidence disproving the role of HPV in urinary bladder carcinogenesis. The point is, according to literature data, that factors determining *INK4a* expression in HPV-associated BC may differ in essence from those in HPV-positive CC. Thus in BC, in contrast to CC, *INK4a* undergoes frequent deletions, point mutations or promoter methylations (Ruas, Peters, 1998; Aveyard, Knowles, 2004; Gallucci et al., 2007). Due to any of these events its expression at the level of protein $p16^{INK4a}$ may become partly or fully lost. For example, homozygous *INK4a* deletions depriving cell of $p16^{INK4a}$ synthesis were found in ~ 30-50 % of BC specimens (Aveyard, Knowles, 2004; Gallucci et al., 2007). Thus our data might prove unsuitability of $p16^{INK4a}$ for role of the HPV-associated BC marker.

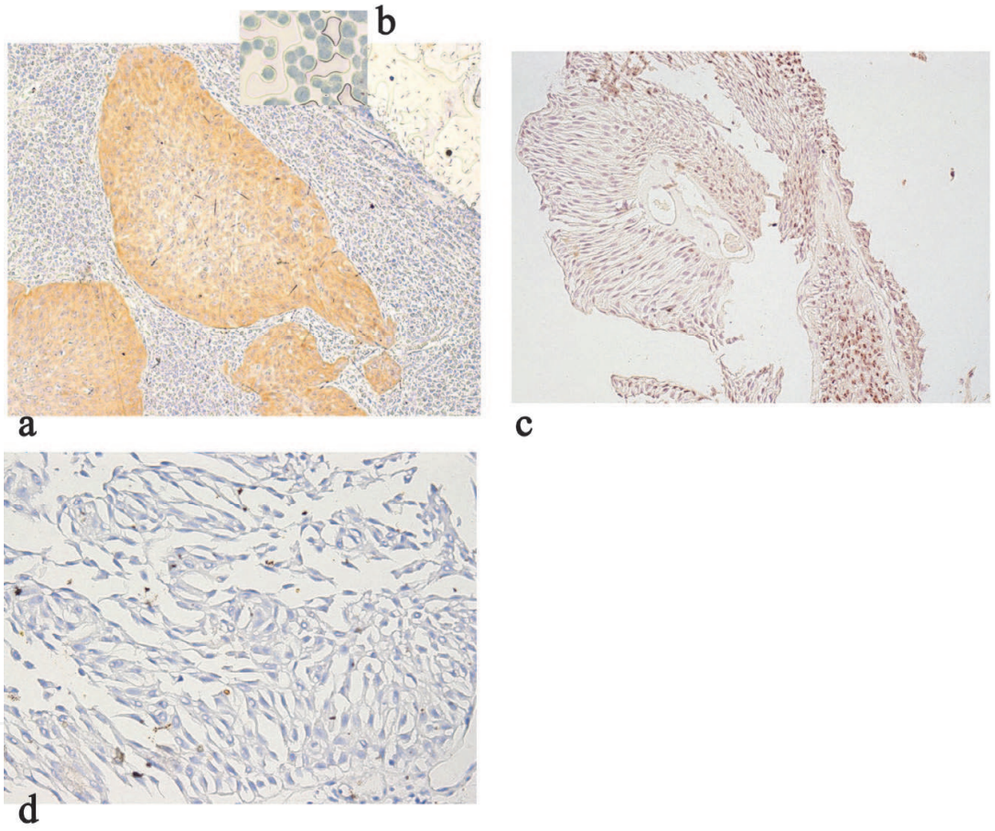

Fig. 3. Results of the immunohistochemical $p16^{INK4a}$ detection in BC specimens.
a. Positive control: HPV16 – harbouring cervical cancer, diffuse staining.
b. Negative control: cells of HCT line (smear), negative reaction with $p16^{INK4a}$-specific antibodies.
c. BC, diffuse staining.
d. BC specimen represented as N3 in Table 1 and "c" in Fig. 1, negative reaction with $p16^{INK4a}$-specific antibodies.

5.3 Summary

The results obtained in two independent samplings of urothelial dysplasia and BC from Russian patients show as a whole that HR-HPV DNA-positivity reaches up to ~ 40-50 %.

Presence of viral DNA in cancer cells is frequently accompanied by expression of viral oncogenes. These results are in agreement with the notion that HR-HPV may take part in BC initiation either solely or in combination with other factors, in particular chemical carcinogens. There are certain reasons still to assume some difference in the action of these viruses in urothelium in comparison with their manifestation in cervical epithelium.

6. Have there been any attempts to investigate the role of papillomaviruses in urothelial carcinogenesis in experimental models?

Urinary bladder similarly to other parts of urinary system (renal pelvis, ureter, etc.) is lined with epithelium of a special kind, the so-called transitional epithelium (Henle epithelium). The question is of particular interest in this connection whether HPV can cause oncogenesis in urinary bladder lining.

M. Campo and co-authors addressed this problem in vivo in cattle (Campo et al., 1992; Campo, 2002). The investigators demonstrated that bovine papillomavirus BPV-2 takes part in BC development under both spontaneous and experimental infection. An important peculiarity of their model is that BPV-associated BC develops commonly in animals being fed with a certain kind of plant, namely bracken fern. Besides BC these animals are affected often with carcinomas in various segments of gastrointestinal tract. When studied particularly bracken fern appeared to contain a number of ingredients which possess mutagenic, carcinogenic and immunosuppressive activities.

The thesis of a species-specific character of papillomavirus infection is well-known (IARC, 2008). In view of this point an exact extrapolation of the data by M. Campo et al. to human papillomaviruses and their possible role in human urothelial oncogenesis seems not quite correct. There are yet some indirect evidences that such extrapolation is not fully groundless. They are as follows. First of all, these researchers found among various histological BC types substantial quota of transitional carcinomas, the type of BC predominating among human patients in many countries including Russia. Secondly, bracken fern similarly to cattle promotes in a human organism carcinogenesis just in gastrointestinal tract. In the regions where it is consumed as food (Brazil in particular) HPV16 is commonly found in dysplasia and carcinoma specimens of esophagus (Campo et al., 1999, as cited in Campo, 2002). Thirdly, transactivation of HPV16 promoter was achieved in experimental model by quercetine, one of the mutagenic ingredients of bracken fern; in such a way it was demonstrated that some types of human cancer in which HPV are being regularly detected may be aetiologically similar to corresponding cancer types of cattle (Campo et al., 1999, as cited in Campo, 2002).

C. Reznikoff and co-authors carried out study on HPV oncogenicity in human urothelial cells in vitro (Reznikoff et al., 1994). The authors transformed isogenic mucosal cells of ureteral uroepithelium obtained from a healthy donor by HPV16 *E6* and/or *E7* gene(s). Cellular immortalization occurred after the integration of either of these viral oncogenes into host chromosomes. Simultaneous integration of both of them led to similar effect. Phenotypic and genotypic alterations were more prominent in cells immortalized by *E6* alone or in combination with *E7* than in cells harbouring sole *E7*. Neither of the transformed cell clones formed tumors when inoculated into nude mice. Some chromosomal alterations found in the transformed cells were identical to karyotype abnormalities found by other researchers in clinical specimens from BC patients. The authors inferred that the phenomena taking place in vitro may correspond to initial stages of urothelial oncogenesis in vivo.

Thus the results obtained in experimental models show that there is no good cause to eliminate papillomaviruses from the list of potential carcinogens in urinary bladder urothelium of Homo sapiens.

7. What benefits may it bring to practical oncourology provided that a certain role of HPV in BC is accepted by medical community?

If this notion is accepted new prospects for BC prevention may come to light. Keeping in mind that efficient vaccines were designed for CC prevention, on the one hand, and that BC is a predominantly male type of cancer, on the other hand, both girls and boys vaccination might become one of such prospects. It is noteworthy in this connection that when the item of reasonability of boys' vaccination is being discussed it is usually being done for the sake of CC prevention in their wives-to-be. Resolution is usually made in the negative in resource-constrained countries. As to the female BC, possibility to prevent women from urothelial carcinogenesis might become an additional convincing argument in favour of their vaccination.

Possible ways of HPV ingress into human urinary bladder lining should be thought over by both clinicians and experimenters. The idea of HPV-associated BC may form grounds for adding of some tests (aimed to detect anogenital HPV) to the currently accepted ways of preoperative check-up of BC patients. This idea may also become the reason to reconsider safety of cystoscope and catheter in treatment of BC patients infected with HPV in anogenital region.

Despite that HPV role in urothelial carcinogenesis is still open-ended question several research groups tried to find an answer to the related one: whether clinical course of BC is affected by HPV presence in urothelial cells.

Y. Andreeva and co-authors studied if papillomaviruses influence relapse incidence in BC patients (Andreeva et al., 2008). The authors preselected 44 BC specimens taken from patients with superficial tumors (stages Ta and T1) on the basis that there occurred koilocytes in these specimens (an indirect morphological sign of viral infection). The specimens were then subdivided into 3 groups: (1) 16 ones from patients with high relapse incidence, (2) 13 - from patients with moderate and (3) 15 - from patients with low relapse incidence. DNA of HPV16 and HPV18 was found by *in situ* hybridization in specimens from patients of the first and second groups only. Seven out of 16 specimens (44%) harboured HPV16 DNA in the first group. Three specimens (23%) were HPV18-positive while HPV16 genetic material was found in neither case in the second group. The authors concluded that HPV occurrence in urothelial cells increases the risk of a superficial BC relapse.

A. Lopes-Beltran and co-authors studied whether HPV DNA presence in cancer cells may influence BC patient survival (Lopes-Beltran et al., 1996). The group of 76 BC patients with transitional BC was formed without any preselection. In materials obtained at transurethral resections the authors detected DNA of HPV6, HPV11, HPV16 and HPV18 using PCR. Follow-up lasted for 5 years. The resultant survival among HPV-positive patients was found to be ~ 29 % (2 out of 7), while among negative ones - 75 % (52 out of 69). The authors concluded that HPV-DNA-positivity serves as a negative predictor of BC patient survival.

The results reported by C. De Gaetani and co-authors (De Gaetani et al., 1999) are in good agreement with those data. The authors found by *in situ* hybridization with the probes to

viral types 16/18 and 31/33/35 HPV DNA in 17 out of 43 BC specimens. Follow-up lasted for 72 months. During this time 10 HPV-positive patients died (~59%). Meanwhile 5 out of 26 HPV-negative patients died (~20%).

If HPV contribution to urinary bladder carcinogenesis gains recognition current therapeutic methods might be supplemented in the near future with the administration to the bladder of HPV-positive BC patients of low molecular weight chemical substances inhibiting HPV oncogenes expression. Results of successful studies of such substances in experimental models were presented at the 25-th International papillomavirus conference (Hellner et al., 2009).

8. Conclusion

The problem of HPV involvement in urinary bladder carcinogenesis is still open. In a complex study performed on clinical specimens of dysplasia and carcinoma of urinary bladder from Russian patients with the use of several methods of HPV DNA detection (*in situ* hybridization, PCR with several types of primers) we registered up to 40-50 % of DNA HPV-positive cases. In many cases DNA HPV-positivity was accompanied with expression of viral oncogenes *E6* and *E7* at the levels of mRNA and/or protein. Thus we detected oncoprotein E7 HPV16 known for its ability to interfere with the normal pRb functioning (which leads to unchecked transition of a cell from G1 to S stage of the cell cycle) in every third BC specimen harbouring HPV16 DNA. Results reported by other research groups obtained both in clinical materials and in experimental models *in vivo* and *in vitro* confirm the idea of HPV as a possible causative agent of BC. There are certain signs that role of HPV in urinary bladder carcinogenesis may be somewhat different from their role in CC origination. Their most probable role in urothelial carcinogenesis seems to be partnership in initiation of the process jointly with other agents (such as parasitic helminths, components of cigarette smoke, chemical pollutants of industrial origin, etc.). The notion that HPV in some cases takes part in urinary bladder carcinogenesis may be helpful for BC prevention, prediction of its clinical course and, in prospect, for treatment of HPV-associated BC.

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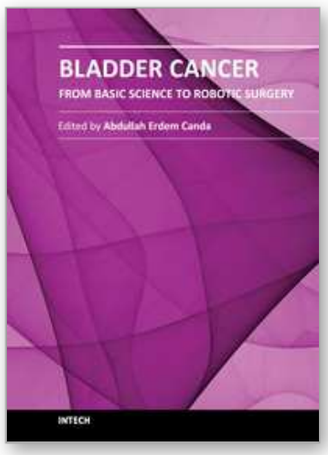

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This book is an invaluable source of knowledge on bladder cancer biology, epidemiology, biomarkers, prognostic factors, and clinical presentation and diagnosis. It is also rich with plenty of up-to-date information, in a well-organized and easy to use format, focusing on the treatment of bladder cancer including surgery, chemotherapy, radiation therapy, immunotherapy, and vaccine therapy. These chapters, written by the experts in their fields, include many interesting, demonstrative and colorful pictures, figures, illustrations and tables. Due to its practicality, this book is recommended reading to anyone interested in bladder cancer.

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