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Review Letter

Genetic alterations by human papillomaviruses in oncogenesis

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The integration sites in the cellular genome of human papillomavirus are located in chromosomal regions always associated with oncogenes or other known tumor phenotypes. Two regions, 8q24 and 12q13, are common to several cases of cervical carcinoma and can have integrated more than one type of papillomavirus DNA. These two chromosomal regions contain several genes implicated in oncogenesis. These observations strongly imply that viral integration sites of DNA tumor viruses can be used as the access point to chromosomal regions where genes implicated in the tumor phenotype are located, a situation similar to that of non-transforming retroviruses.

Papillomavirus; Oncogen; Tumor phenotype

1. INTRODUCTION

The oncogenic process is the result of the sequential accumulation of mutations, some of which are selected for the new biological properties of the affected cell. These mutations will result in the development of a tumor and more importantly lead to the appearance of tumor cell variants that give rise, among others, to metastasizing cells. The mutations are variable and can range from point mutations like in the *ras* gene family [1] to gross chromosomal aberrations like translocations or deletions [2]. In many cases the mutation observed is a characteristic of a specific type of tumor, but this does not imply that it is the only one. At least six different genetic alterations have recently been found in colon carcinoma [3]. In different tumors the nature of the mutagenic agent may be different but not its consequences.

About twenty percent of human tumors are associated with the presence of a virus [4]. Human papillomaviruses (HPV) are linked to the development of cervical carcinoma and some skin carcinomas [4,5]. On a world-wide basis cervical carcinoma is the second type of cancer in women [5,6]. In advanced stages of cervical carcinoma a common observation is the integration of the viral DNA into the cellular genome, but its role is poorly defined. This integration is not part of the life cycle of the virus, however it is a clonality marker of the individual tumor. The integration of these viruses partially destroys the structure of the viral genes, some of which are no longer functional. If these integration sites were selected they might become markers for the location of a chromosomal region where genes implicated in oncogenesis might be located. Nevertheless it is not clear what the contribution of the integration to the tumor phenotype is. One way to provide an answer is by reviewing the location of the integration sites and see whether they are in regions associated with an oncogenic phenotype or a particular type of gene. Tumors associated with DNA tumor viruses have a long latency period which might be the result of the time needed to get integrations in the 'proper' locus for tumor development. The observation of multiple chromosomal regions of viral DNA integration may be a consequence of the multigene process of tumorigenesis and the biological step where integration takes place.

In the better known process of oncogenesis by non-transforming retroviruses, at least twenty genes implicated in oncogenesis are affected by provirus insertion [7,8]. Another twenty chromosomal regions have been identified as common regions of provirus integration, some of them are implicated in the late stages of oncogenesis like tumor progression, metastasis, and in the selection of specific tumor cell subpopulations for reasons not yet identified [7,8] In these tumors there is always a long latency period and in order to identify these loci many tumors have to be examined before they are detected as common integration sites. To understand the action of the provirus mechanistically it is important to notice that a provirus can alter the regula-

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tion of a gene at a distance greater than 250 kb [9] and that a single provirus can affect several genes on the same chromosome [9,10]. Conceptually from a biological point view, integration of an RNA or DNA tumor virus can have the same effects although their integration mechanisms are different.

INTEGRATION REGIONS OF HUMAN PAPILLOMAVIRUSES DNA

Human papillomaviruses form a group of viruses with more than sixty different types based on their nucleotide sequence. These types have different properties based on their oncogenic potential and the type of epithelium where they are more frequently localized. Only four viral types, HPV-16, HPV-18, HPV-31 and HPV-33, are associated with over ninety percent of cervical carcinomas [11].

The integration of HPV DNA is usually detected in advanced cancers. Nevertheless, in one case the integration was already detected in a premalignat lesion [12], perhaps because the integration in the oncogenic location took place early on and was subsequently selected. In very few carcinomas related to HPV the integration of the viral DNA has been studied. Fortunately the mapping of the integration sites has been performed in a sufficient number of cases to be able to draw some conclusions [12–17]. Also, several cell lines derived from cervical carcinoma have been established and the chromosomal location of the integrated viral DNA determined [13,15,18–24]. The data is summarized in Table I. All the integrated viruses have in common their location on chromosomal regions known to contain an oncogene or being associated with some other tumor pathology. The most striking observation, particularly in view of the very few cases studied, is the detection of two chromosomal regions, 8q24 and 12q13, with integrated viral DNA of two different types, HPV-16 and 18, in several cases.

8q24 IS A COMMON INTEGRATION SITE

The chromosomal region 8q24 contains at least four loci implicated in tumorigenesis: the oncogen c-*myc*, and the loci *pal*-1, *mlvi*-1 and *mlvi*-4. The locus *pal*-1 codes for 5 and 10 kb messages (E.F., P.A.L., unpublished) and is expressed in several tumor cell lines [19,24]. The *mlvi*-1/*pvt* [9] and *mlvi*-4 [10] loci, initially identified as common regions of provirus insertion, are located 300 and 30 kb 3′ of c-*myc*, respectively. The *mlvi*-4 locus codes for two messages of 3 and 5.5 kb which are lymphoid specific [10]. However no precise mapping of the integration site or study of the possible functional alterations of these genes has been done.

DNA of two viral types, HPV-16 or 18, has been detected in the chromosomal region 8q24. The tumor phenotype is variable and includes two cervical adenocarcinomas [15], one penile carcinoma [15], the Hela cell line [13,24], derived from a cervical adenocarcinoma, and an argyrophhyl cell carcinoma [17]. This last type is particularly agressive.

In all the cases of HPV integration in 8q24 there is an amplification of the viral DNA sequences which ranges from 5 to 50 copies and are usually forming a head to tail array. On the other hand the status of the c-*myc* gene is very variable. In only one case there is amplification of c-*myc* [15]. With the exception of the penile carcinoma there is no rearrangement of the c-*myc* gene [15]. This observation implies that the integration takes place at a significant distance from the c-*myc* gene. In two cases there is overexpression of c-*myc*, but this overexpression has no correlation with the level of HPV DNA amplification. A threefold increase in expression is detected whether there is no integration, or the amplification ranges from five to fifty copies of integrated viral DNA [13,15,17,24]. In the Hela cell line there is a light increase in *myc* RNA levels line consistent with its constitutive expression [13,24]. Further studies promise to shed more light on the role of the 8q24 region in human oncogenesis. The lack of a correlation between the integration/amplification of HPV DNA and c-*myc* gene makes it unlikely that the effect on c-*myc* is the main reason for the selection of these integrations. Obviously c-*myc* does play a role, like in many other tumors, but this is in addition to the effect of the viral integration in the same chromosomal region. A candidate gene might be located in the *pal*-1 locus, the integration site of HPV-18 DNA in Hela cells [19,24], from which two messages have been detected in some tumor cell lines like Colo320 [24], HL-60 [24] and some Burkitt's lymphomas (E.F. and P.A.L., unpublished).

Other lines of research also support the involvement of the chromosomal region 8q24. In experiments involving the immortalization of human keratynocytes with HPV-16 there is an amplification of the c-*myc* region without rearrangement of this gene [25]. In this cell line, W12, the integrated viral DNA is amplified to the same extent as c-*myc*, but no linkage was established between the two [25]. Some other reports have been published describing structural alterations 5′ from c-*myc* in papillomavirus induced tumors, but no correlation was done regarding the integration of the virus [25,26]. In animal models, like transgenic mice with BPV-1, viral integration have been found in mouse chromosome 15 which, interestingly enough, is syntenic to human chromosome 8 [27].

12q13 IS ANOTHER COMMON REGION OF INTEGRATION

The other common chromosomal region of integration, 12q13, is also a region associated with a large variety of tumors [28]. In this chromosomal region there are three known oncogenes, *int*-1, *gli*-1, a member of the

Kruppel family, and *rap*1B, a member of the *ras* family [28]. Another interesting gene in the region codes for the melanoma antigen ME491 [29,30]. This antigen belongs to a family of genes with antiproliferative properties [30], thus melanomas with high levels of this antigen have a much better prognosis than those with low levels of ME491 [29]. These antigens are expressed in tumors of epithelial origin which makes this antigen a possible candidate for alterations in cervical carcinoma. Nevertheless this possibility has yet to be explored. Two cell lines, SW756 [20] and SK-v [14], have integrated HPV-18 and HPV-16 DNA, respectively. The genes *int*-1 or *gli*-1 are not affected by the integrated viral DNA in the case of SK-v cell line [12], and they have not been studied in the case of SW756 cells. In the SK-v cell line this integration was already present in the early stages of the original tumor, the premalignant lesion, suggesting that it might play a role that early in the process [12]. In either case, we do not know what the proximity of HPV sequences to these oncogenes is. It is likely that other tumorigenic genes, not yet identified, are located in this chromosomal region.

Table 1

Human papillomavirus integration regions

Tumor or cell line (CL)	Viral type	Chromosomal location	Genes	Other tumor phenotypes linked to this region
Squamous cell carcinoma [15]	HPV-18	2p24	N-*myc* FRA2C	Neurobiastoma Lung carcinoma
Cervical carcinoma [17]	HPV-18	3p21-22	*erb*A2	Non-Hodgkin's lymphoma, pleomorphic adenoma, adenocarcinoma (kidney, ovary), lung carcinoma
Cervical carcinoma	HPV-16	3q25	*raf-1*	
Hela (CL) [13,16,21]	HPV-18	5p11-15 abnormal	FRA5A and B, *mlvi*-2, GHR	Adenocarcinoma
C4-1 (CL) [18,23]	HPV-18	8q22	*myb*-B FRA18A and B	Acute myeloid leukemia
Cervical adenocarcinoma [15]	HPV-18	8q24	c-*myc*, *pal*-1, *mlvi*-1 and 4, FRA18C, D and E	Burkitt's lymphoma, acute lymphoblastic leukemia, malignant lymphoma, familial renal carcinoma
Cervical adenocarcinoma [15]	HPV-16	8q24	ibid.	ibid.
Penile carcinoma [15]	HPV-16	8q24	ibid.	ibid.
Argyrophil small cell carcinoma [17]	HPV-16	8q24	ibid.	ibid.
Hela (CL) [13,16,21,24]	HPV-18	8q24	ibid.	ibid.
Hela (CL) [13,16,21]	HPV-18	9q31-q34	c-*abl*, FRA9B and E	AML, CML, ALL
SK-v (CL) [14]	HPV-16	12q13	*int*-1, *gli*-1, ME491, *rap*1B, FRA12 A, homeo box 3	Melanoma, lipoma, liposarcoma, glioma, leiomyosarcoma, pleomorphic adenoma, T-cell lymphoma
SW 756 (CL) [20]	HPV-18	12q13	ibid.	ibid.
SiHa (CL) [21]	HPV-16	13q21-31	FRA13B, C, excision repair (ERCC5)	
Hela (CL) [13,16,21]	HPV-18	22q12-q13 abnormal	PDGF, V-*ves* homolog, NF2, LIF, MGCR, *bcl*-2, FRA22A	Neurofibroma, meningioma, Ewing's sarcoma, CML

The data on gene location is based on the 10th Human Gene Mapping Conference [28].

Table II

Function of genes located in chromosomal integration sites of human tumor viruses

Growth factors:	
IGF-2	FSH
PDGF	*int*-1
LIF	*int*-2
interferons	*hst*
Growth factor receptors/tyrosine kinases:	
Retinoic acid receptor	c-*abl*
Progesterone receptor	c-*src*
Thyroid hormone receptor	*yes*
Growth hormone receptor	
Signal transduction:	
phospholipase C	*rap*1B
NF-2	*mos*
protein kinase C	*erb*A2
raf-1	
Membrane proteins:	
ME491	Thy-1
CD44	CD3
CD5	CD20
Nuclear proteins/transcription factors:	
c-*myc*	*gli*-1
N-*myc*	*ski*
ets-1	*elk*-1
cyclin A	
Proteases:	
stromelysin	
collagenase	
Tumor suppressor genes:	
p53	MEN-1
WT-1	
Other loci:	
mlvi-1	*bcl*-1
mlvi-4	*bcl*-2
pal-1	CBL-2
MGCR	

RELATION WITH THE INTEGRATION SITES OF OTHER HUMAN TUMOR VIRUSES

Hepatitis B virus is linked to the development of hepatocellular carcinoma [31]. Two common regions of integration have so far been detected in hepatocellular carcinoma cases, 11q13 and Xp22, where integrated viral DNA has been found in two tumors (reviewed in [31]). The 11q13 region has several oncogenesis-related genes like *bcl*-1, *hst*-1, *int*-2 and MEN-1 (multiple endocrine neoplasia). The Xp22 region contains the *elk*-1 gene which is related to the *ets*-1 family of transcription factors [32]. The *ets*-1 gene itself, the transcription factor PEA3 [33], is the target of HBV integration in another tumor. There is also a chromosomal location, 12q13, which is also the integration target of hepatitis B virus [31] and HPV [14,20], thus two types of viruses can be found in this interesting chromosomal region.

Based on the data on HPV (this report) and HVB [31] the frequency of finding a common chromosomal alteration in either cervical carcinoma or hepatocellular carcinoma is in the range of 10–20 percent of the cases. This frequency is the same found for many loci implicated in oncogenesis by non-transforming retroviruses [8], and suggests that all of them might play a similar role in the pathogenesis of the disease.

FUNCTION OF GENES AFFECTED BY DNA VIRUS INTEGRATION

In all the cases studied, the location of the integrated papillomavirus DNA (Table I) or hepatitis B virus DNA [31] correlates very well with either chromosomal regions associated with other tumor pathology or with chromosomes fragile sites [2,23].

We can look at the integration sites from the point of view of the function of the genes located in these chromosomal regions (Table II). The putatively affected genes can be functionally classified in several categories like genes coding for growth factors, growth factor receptors, membrane or cytoplasmic proteins involved in signal transduction, and nuclear proteins most of which are known to have transcription factor activity. Also other genes potentially implicated in the dissemination of the tumor, like proteases, might be affected. In the data shown in Table II there are twenty-five oncogenes, three supressor genes and several other genes the function of which is related to that of known oncogenes, like several growth factor receptors. It is important to notice that these genes are the same that can be affected in oncogenesis by non-transforming retroviruses [8]. Mechanistically the integrated viral DNA is likely to alter these genes in a fashion similar to the non transforming retroviruses implicated in oncogenesis, most likely by an enhancher insertion mechanism [7,8].

CONCLUSION

Human papillomaviruses and human hepatitis B virus by persisting a long time as a chronic infection in epithelial or hepatic cells, respectively, have the potential for occasional integration in the cellular genome. Some viral DNA integrations might be selected for the consequences on the tumor phenotype, which might be an early or late tumorigenic event. It is our view that integrated HPV and HBV DNA are markers for the location of genes implicated in the tumor phenotype, in a way similar to the non-transforming retroviruses. All the chromosomal integration regions so far identified with both viruses are consistent with this view. Taking into account the small number of cases studied in both diseases, it is highly likely that these common integration sites, either for one type of virus or both, are the result of strong biological selection for some properties of the tumor cell which remain to be discovered. Integrated viral DNA can thus be an important tool in the

search of human genes related to oncogenesis which otherwise would be extremely difficult to identify.

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