World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i42.6027

World J Gastroenterol 2012 November 14; 18(42): 6027-6035
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Human endogenous retroviruses and cancer: Causality and therapeutic possibilities

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Author contributions: Mullins CS and Linnebacher M analyzed the literature and wrote the paper.
Supported by Grants from the State Mecklenburg-Vorpommern and from Deutsche Krebshilfe, No. 108446
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Received: June 5, 2012 Revised: September 10, 2012
Accepted: September 19, 2012
Published online: November 14, 2012

Abstract

A substantial part of the human genome is derived from transposable elements; remnants of ancient retroviral infections. Conservative estimates set the percentage of human endogenous retroviruses (HERVs) in the genome at 8%. For the most part, the interplay between mutations, epigenetic mechanisms and post-transcriptional regulations silence HERVs in somatic cells. We first highlight mechanisms by which activation of members of several HERV families may be associated with tumor development before discussing the arising chances for both diagnosis and therapy. It has been shown that at least in some cases, tumor cells expressing HERV open reading frames (ORFs) thus gain tumor-promoting functions. However, since these proteins are not expressed in healthy tissues, they become prime target structures. Of potential pharmacological interest are the prevention of HERV transposition, the inhibition of HERV-encoded protein expression and the interference with these proteins' activities. Evidence from recent studies unequivocally proves that HERV ORFs represent a very interesting source of novel tumor-specific antigens with even the potential to surpass entity boundaries. The development of new tumor (immune-) therapies is a very active field and true tumor-specific targets are of outstanding interest since they minimize the risk of autoimmunity and could reduce side effects. Finally, we postulate on main future research streams in order to stimulate discussion on this hot topic.

Key words: Human endogenous retroviruses; Gastrointestinal cancer; Therapeutic targets; Tumor-specific antigens; Tumorigenesis

Peer reviewers: Yujin Hoshida, MD, PhD, Cancer Program, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, United States; Atsushi Nakajima, Professor, Division of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan

Mullins CS, Linnebacher M. Human endogenous retroviruses and cancer: Causality and therapeutic possibilities. *World J Gastroenterol* 2012; 18(42): 6027-6035 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i42/6027.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i42.6027

HUMAN ENDOGENOUS RETROVIRUSES: AN INTRODUCTION

Human endogenous retroviruses (HERVs) are remnants of ancient retroviral infections. Many insertions into the genome have taken place tens of millions of years ago[1,2]. Since the first set of data on the human genome project has been published in 2001, it is well established that about 8% of the genome consists of HERVs and their total number is approximately 3×10^5 copies[3]. Generally, HERVs are classified into three groups:

class I [gamma (like) retroviruses], class II [beta (like) retroviruses] and class III [spuma (like) retroviruses][1]. The most common nomenclature utilizes the single-letter amino acid code corresponding to the tRNA primer that is used for reverse transcription of the HERV genome[4]. For the most part, their canonical structure of a single open reading frame (ORF) consists of the *gag*, *pol* and *env* genes flanked by 5' and 3' long terminal repeats (LTR)[2,5]. The latter features are what endogenous and exogenous retroviruses have in common.

ENDOGENIZATION

Following a retroviral infection, the fate of the host depends on the pathogenicity of the infectious virus. Highly pathogenic ones will kill the host whereas ones with only weak pathogenicity may manage to infect many different cell types, including reproductive tissue cells. Subsequently, an endogenous retrovirus will establish if virus and host proceed to fixation of the virus' sequences in the host genome. This process has been termed endogenization or molecular domestication[6]. In most cases, HERV activities have been silenced by a variety of mechanisms. Mutational inactivation includes deletions as well as point mutations and probably has been triggered by specific regulatory proteins such as APOBEC[7,8]. Furthermore, epigenetic mechanisms including methylation and histone modification contributed to HERV inactivation[9]. Besides directly silencing the expression, posttranscriptional regulation further protects the host's genome[10]. It has been argued that the presence of such a high number of HERV copies must be advantageous for the host, too[11]. An amazing idea suggests that over time of evolution, retroelements like HERVs actively contribute to the development of novel physiological capacities[12,13]. It is for example easily imaginable that a *de novo* ORF which basically encodes a membrane protein may give rise to a protein with novel functions when mutated. If such a protein is beneficial to the host, it will be fixed. Thus, HERVs are together with other mobile genetic elements drivers of the (human) evolution by providing material for genomic evolution, variation and natural selection[6,12,14]. This argumentation adds another level of complexity to the relationship of humans (and all other vertebrates) and retroviruses[15]. Consequently, HERVs must not be considered as parasites but as true symbionts - on the population level. However, the individual risk for *de novo* insertions is rather low with estimated rates of only 1 in 100 births[16].

PRESERVED FUNCTIONS

Although the vast majority of HERV sequences have been inactivated over time as outlined above, there are some examples of HERVs with potentially useful functional modules; comparable to the proviruses of their exogenous counterparts. Among the cellular functions influenced by HERVs are enhancement and promotion of gene expression. In a study on primate evolution ERV-9 LTR sequences were found in higher primates and humans. In the latter, tissue specific enhancer activity could be detected in hematopoietic cells and even stronger in embryonic cells[17]. HERV-E LTR functions as enhancer for endothelin B receptor and apolipoprotein C- I genes in humans[18]. Furthermore, HERV sequences also give rise to novel or alternative splicing and polyadenylation sites[19]. They also can be involved in membrane fusion, with Syncytin in the placenta being the most prominent representative here of[20].

HERV AND CANCER

A variety of oncogenic mechanisms have been attributed to animal oncogenic retroviruses[21,22]. Moreover, it has been suggested, that failures and errors in single somatic cells' efficiency to control HERV activity potentially results in genome damage and may thus contribute to the formation of cancer[14]. The possible oncogenic mechanisms of HERVs include (Figure 1): (1) the general or more specific (re)activation of HERV sequences by hypomethylation[23-25]; (2) the expression of HERV encoded oncogenes such as Rec and NP9[26]; (3) the inactivation of tumor suppressor genes by *de novo* insertion or translocation of retroelements within the genome[26]; (4) the regulation of nearby (proto-) oncogenes or growth factors by the regulatory sequences of LTRs[27,28]; and (5) the potential of Env proteins to induce cell fusions, which may contribute to tumor progression or even aid in metastasizing processes[29]. Far from being complete, this is already a quite impressive list. An additional aspect comes from the observation that Env proteins of the mouse leukemia virus, the Mason-Pfizer monkey virus and also of HERV-K have strong immunosuppressive properties and may thus help tumor cells evade an antitumoral immune response[26,30,31].

Methylation

As a general rule, all human regulatory genomic sequences become methylated unless specific factors prevent methylation[23]. In addition, methylated sites are more prone to mutations[23] and by this means, virus inactivation is further strengthened. Demethylation of regulatory regions is possible in the context of normal physiological processes by strong transcriptional activators. Re-expression of methylated sites is also possible during cell stress dependent on chromatin remodeling as a reaction to this stress[32]. Obviously, the maintenance of methylation patterns and status must play a central role in HERV transcriptional control. In healthy somatic and mature germ cells HERV sequences are generally (hyper-) methylated. Thus, HERV transcriptional activity is mainly restricted to germ cell development or the desensitization of check-point activation in meiotic cells. This mechanism may also be responsible for a high(er) retroelement expression in germ cell tumors[23]. In somatic cells, severe global hypomethylation

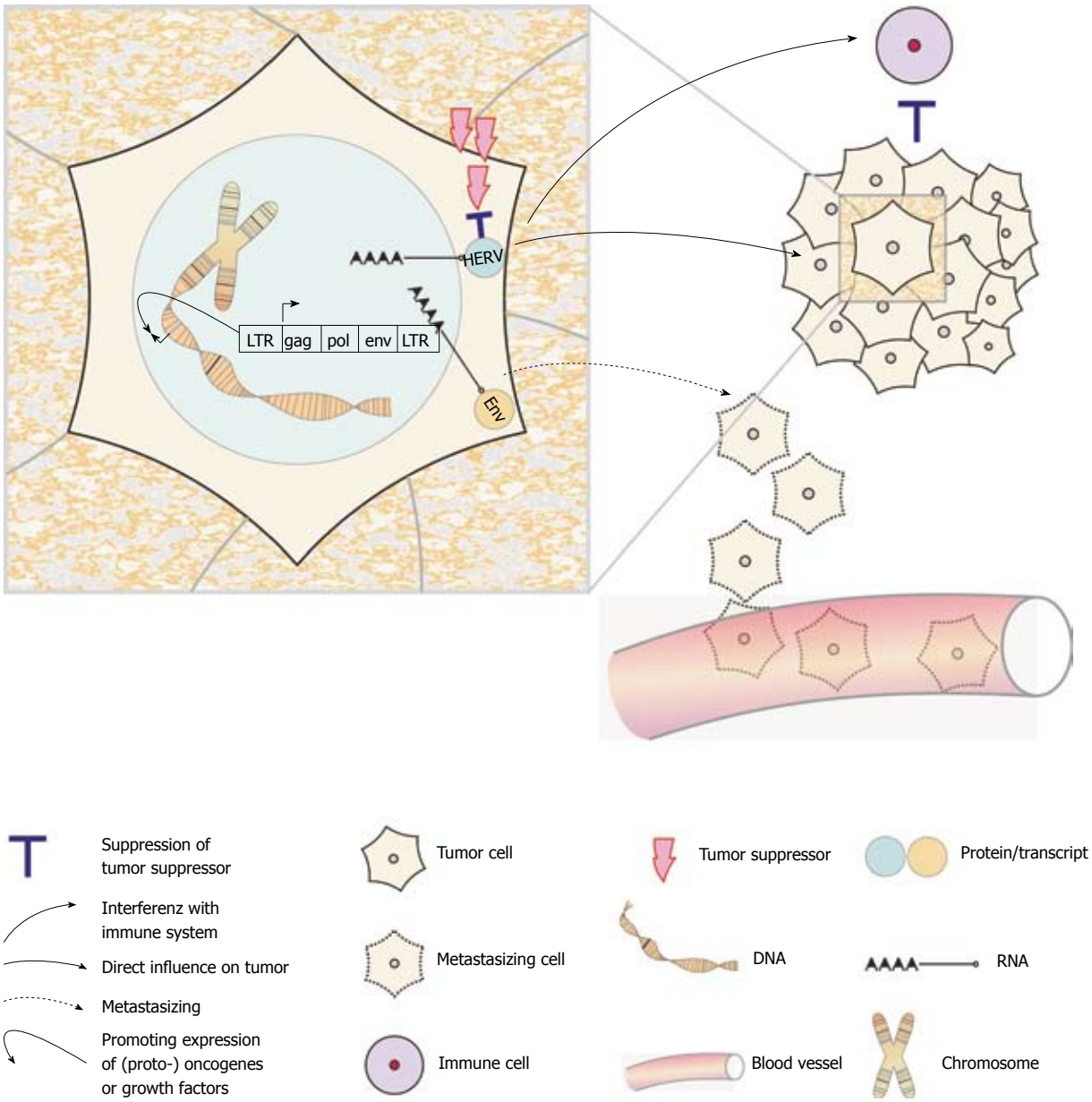

Figure 1 Possible mechanisms by which human endogenous retroviruses contribute to oncogenesis. Human endogenous retroviruses (HERVs) transcripts or proteins may directly have tumor promoting properties. The long terminal repeat (LTR) elements can function as promotors or enhancers for nearby (proto-) oncogenes or growth factors. Especially Env proteins might attract regulatory immune cells and thus provide an immunosuppressive microenvironment. And finally, the Env proteins may be directly involved in the metastasizing process.

leads to apoptosis induction mediated by TP53 and other tumor suppressive factors[33]. Premalignant and malignant cells are typically insensitive to apoptosis induction[34] and aberrant expression from normally methylated promoters is a main oncogenic force. In line with this, a general hypomethylation of HERV sequences can be found in the cancer cells of different entities, including testicular germ cell cancer, teratocarcinomas, colorectal, breast and ovarian cancer[35-39]. However, methylation analyses are biased by a lack of accuracy of the bisulfide sequencing technique[40]. When not highly standardized, this may account for a number of false positive or negative results in methylation analyses. Thus, it is always recommended to combine methylation analyses together with an investigation of mRNA or superior protein expression.

HERV ORF expression

Of interest, Syncytin-1 is the only expressed HERV sequence with a presumable physiological function. Syncytin-1 expression takes place in the placenta in the context of syncytiotrophoblast generation by cellular fusion of precursor cells, the cytotrophoblasts[41,42]. This expression follows after a general hypomethylation of a HERV-W *env* sequence and the Env protein is considered to contribute to this cellular fusion process. It may be a coincidence, but for many tumor entities, naturally occurring cellular fusions have been described[43-45] and this may hint towards the expression of similar HERV Env proteins.

Expression of HERV sequences has been described for several tumor entities including melanoma, breast, ovarian, prostate and colon cancer[37-39,46,47]. Active retrotranspositions cause DNA strand-breaks and will thus lead to an activation of check-point signaling, e.g., TP53. Thus, transpositions as another mechanism for HERV re-expression may consequently occur especially in tumors with defect check-points and TP53 mutations[23].

Tumor induction/promotion

Beside sheer tumor specific expression, HERVs have repeatedly been discussed to induce or promote tumorigenesis. Potential mechanisms have been outlined in the preceding paragraphs. Here we want to gather the bits of evidence that have been obtained so far.

Several groups could show the production of HERV-derived proteins or even of viral particles in tumor cells[48-54]. In a mouse study, Howard and coworkers could directly link genome hypomethylation to ERV up-regulation[55]. Further research could make the connection between hypomethylation of (H)ERVs and chromosomal instability; it is by mediating ectopic recombination[56]. These HERV-induced recombination events have been found to produce large scale chromosomal anomalies[57], a hallmark of most tumors[34]. Finally, Lamprecht and colleagues could link the deregulated expression of the colony-stimulating factor 1 receptor (CSF1R) in B cell-derived Hodgkin's lymphoma cells to hypomethylation of an up-stream HERV-derived LTR, which promotes ectopic expression of the CSF1R proto-oncogene[58]. However, the question if the reactivation of a (pro-) virus could actively promote cellular transformation or at least contribute to tumor progression is formally unsolved for human cancer. Similarly, it is unknown, if HERV activation is an early or a late step in tumor formation. Still, when considering the above listed bits of evidence, it seems reasonable to conclude that HERVs' contribution to the multi-step process of tumor development in humans is very likely[14].

Immune responses towards HERV sequences

The human immune system's capability to recognize HERV sequences has so far only scarcely been analyzed. However, some examples can be found in the literature. In patients with kidney cancer, cytotoxic T lymphocytes (CTLs) reactive to a HERV-E sequence encoded on chromosome 6q were found[59]. Serological responses and CTLs reactive to HERV-K sequences were detected in melanoma patients[60,61]. Anti-Env antibodies for HERV-K, -E and ERV3 were present in sera of patients with ovarian cancer[38] and in male patients with germ cell tumors[62]. Similarly, in breast cancer patients, anti-HERV-K serum antibodies were detected together with HERV-K-specific CTLs[63]. The orchestrated activation of both arms of the adaptive immune system in the latter cases is a strong indicator of HERV sequences' high immunogenicity. Consequently, one may conclude that at least no strong tolerance towards HERV encoded sequences is induced during lymphocyte development. Future studies will have to analyze whether the immunological recognition of HERV sequences is executed by highly avid or only by intermediate avid T cells and antibodies. Also, it must be carefully analyzed, which HERV sequences give rise to strong immune responses when aberrantly expressed in tumor cells. We would like to state that immune recognition is a strong indicator for endogenous expression of a given HERV protein, as has been shown for other tumor antigens[64]. Of note, T cell reactions against HERVs, such as HERV-K, HERV-L and HERV-H, were associated with successful control of human immunodeficiency virus (HIV) in a subset of HIV patients[65]. It can be anticipated that this association of successful HIV control by HERV specific immune reactions will be translated into the tumor field. One of the major questions with clinical relevance is whether HERV-specific immune signatures can be associated with better prognosis or not.

THERAPEUTIC STRATEGIES

Assuming that in the normal physiology of adult tissues, HERVs do not play a vital role and following the line of evidence that HERV sequences are of significance in tumor formation, development and metastasis, HERVs recommend themselves as prime targets for tumor therapy. Several targeting strategies have been suggested (Figure 2 for an overview) and first experimental results can be found in the literature.

Inhibition

In the light of the tremendous success in HIV control with infected people treated by antiretroviral combination therapies, it would make sense to simply reverse the expression of HERV sequences in human tumor cells. The group of Carlini analyzed the effect of a reverse transcription inhibitor (Abcavir) on prostate cancer cell lines[66]. It showed a strong anti-proliferative capacity and even triggered senescence in the cancer cells. Interestingly, the authors found an up-regulation of transcripts from LINE elements in the treated cells but unfortunately, they did not analyze HERV expression[66].

A direct targeting of HERV proteins by small molecular inhibitors or via RNA interference would also be worth trying. However, this has not yet been done. Therapeutical use of natural inhibitors of retroviruses such as APOBEC[67,68] or TRIM5[69] would be another possible future option. First, detail knowledge on how and when such retroviral restriction elements act on HERVs must be build up.

Passive immune therapy

Only very recently, Wang-Johanning and coworkers designed a monoclonal antibody (mAb) recognizing a HERV-K Env protein. They described that HERV-K Env protein expression was substantially higher in malignant breast cancer cell lines than in non-malignant breast cells. Furthermore, HERV-K expression was detected in 148

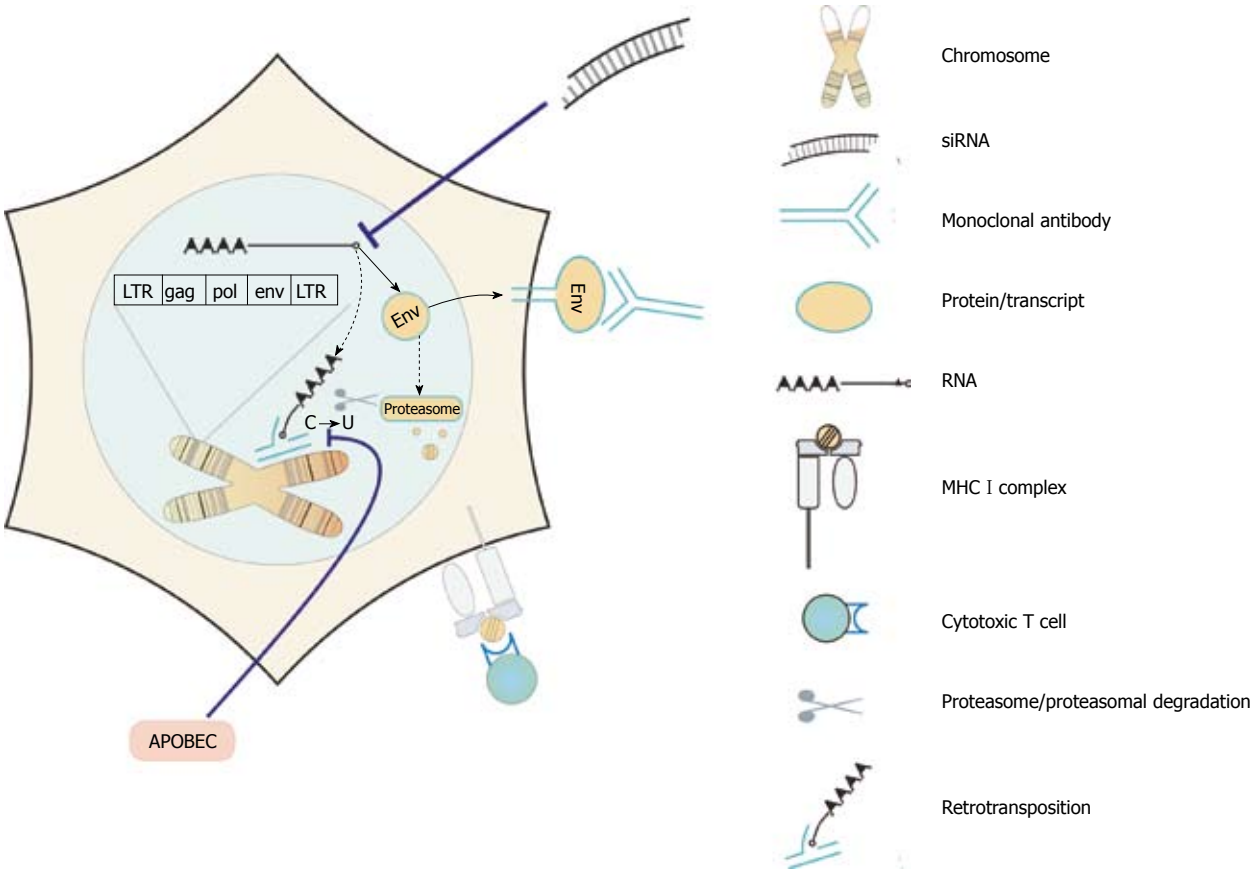

Figure 2 Therapeutic possibilities to target tumor cells with active human endogenous retroviruses. Expressed Env proteins may be targeted by therapeutic monoclonal antibodies. (Re-) activation of retroelement-controlling proteins may help to reduce human endogenous retrovirus (HERV) activities. Small molecule inhibitors of HERV proteins or inhibitory targeting of expressed HERV sequences potentially will prevent oncogenic properties of HERV and harm or kill tumor cells with activated HERV oncogenesis. HERV proteins with tumor-specific antigen properties can be targeted by specific T cells. LTR: Long terminal repeat; MHC: Major histocompatibility complex.

(66%) of 223 primary breast tumors. And a higher rate of lymph node metastasis was associated with HERV-K-positive tumors. Anti-HERV-K-specific mAbs inhibited tumor growth and induced apoptosis of breast cancer cells *in vitro*. Mice treated with these mAbs showed significantly reduced growth of xenograft tumors. *In vitro*, this treatment resulted in an over-expression of several proteins involved in the apoptotic signaling pathways in malignant breast cells[70]. In principle, targeting HERV Env proteins by therapeutical antibodies should be exploitable to all individual tumors expressing HERV Env. Moreover, passive immune therapies may well be applied in combination with active immune therapies.

Active immune therapy

The ideal cancer therapeutic agent should be able to discriminate between cancer and normal cells (i.e., specificity) and be potent enough to kill small or large numbers of tumor cells (i.e., sensitivity). A feature that makes immunotherapies unique is that an ideal cancer immunotherapy should be able to prevent recurrence of the tumor (i.e., durability). In the last decades it became increasingly apparent that this durability in prevention of tumor recurrences is due to persistent recognition of tumor antigens by lymphocytes.

Researchers distinguish between tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). TAAs are antigens that are expressed in normal tissue but to a much higher extent in malignant cells. Contrary to this, TSA are truly specifically expressed in tumor cells alone. Beside specific point[71] or frameshift mutations[72], proteins from tumor-inducing viruses[73] for the most part form this class of tumor antigens. Most of the features an ideal TSA should possess have been assigned to HERV encoded proteins. This being beside exclusivity also the necessity of expression for maintenance of the cancer cells' transformed state. Thus, immune-escape by simple down-regulation of expression is prevented[74]. Moreover, to ease therapy development, ideal TSA expression should be not only present in single tumors but shared between individual tumors of a given entity or even superior between tumors of different entities[75,76]. Finally, the immune system should be able to mount both a cellular and a humoral response[63]. When summing up these desired properties of TSAs attributable to (at least some) HERV-encoded proteins, one may conclude that they might indeed be ideal targets for tumor immunotherapy. Because of the multitude of HERV-

encoded sequences one can even expect that the development of a polyvalent (i.e., containing many epitopes) vaccine basing only on HERV epitopes may be possible. Even more visionary, actual bioinformatics approaches will allow the identification of immunogenic core epitopes shared between different HERV copy ORFs active in different tumor entities in order to design a universal HERV-based vaccine. As a first step in that direction, we recently described two $CD8^+$ T cell epitopes encoded by a HERV-H copy located on Xp22.3[77].

FUTURE PERSPECTIVES

At the moment, in the field of HERVs more questions are open than answered. Are there human (tumor-) cells producing virus particles? If so, are those particles infectious? Further analyses on expression of HERV sequences and proteins - and in especially of Env proteins - would add to the full picture and understanding of the relationship of tumors and endogenous retroviruses. In a first step the mutual interaction between HERV Env and the immune system, also in a suppressive manner, has been addressed[26,30,31]. These analyses should be expanded. Especially a broader knowledge on tumor infiltrating cells specific for HERV epitopes and their prognostic value would be interesting. Furthermore, it would be very beneficial to know if there is a correlation between tumor grade, stage, progression or outcome and the expression of HERV sequences.

CONCLUSION

Our understanding of HERVs has come a long way. They must be considered as domesticated retroviruses with even main functions in evolution. On the level of an individual human being, however, their activities most likely are tightly controlled. Heavy genetic disorders, as present in tumor cells, generally seem to be linked with HERV activities. The tumor-specific expression of HERV-encoded proteins opens the way to diagnostically and therapeutically interesting opportunities: (1) The targeting of HERV proteins either biochemically or immunologically as TSAs; (2) Immune recognition of tumor cells takes place already early in tumor development. HERV-encoded ORF-derived proteins are likely candidates of this early recognition. Consequently, they may be ideal for screening people at risk to develop cancer as we suggested for frameshift mutations in lynch syndrome[72,78]; (3) the recognition of expressed HERV sequences by the adaptive immune system is likely to result in a better prognosis for patients raising to-be-defined minimum levels of immune responses. Such HERV-specific responses may well be suited for prognostic purposes.

POSTULATES

We would like to take the chance and hypothesize on some of the open questions and obvious tasks in the HERV/tumor field: APOBEC and other retroelement controlling factors are likely to be inactivated in cancer cells with active HERV-driven oncogenesis. If this is frequently the case, they must be considered tumor suppressor genes and screening for their inactivation would possibly hint towards specific HERV activation.

HERV-encoded TSAs are released into the circulation[79] and thus screening of HERV-TSA blood levels will become an interesting field of investigation. Similarly, HERV-specific (immune-) therapies will be developed in the near future for several tumor entities. For these immunotherapies, beside knowledge about expression in different tumors, the level of tolerance towards HERV-TSAs will guide the decision on which candidates to investigate in clinical trials.

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