Retroviruses and oncogenesis

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Abstract: Viruses and cancers have been important subjects for research in biomedical sciences for many years. It is now estimated that at least 20% of human cancers worldwide have an underlying viral etiology. In this article oncogenesis by retroviruses will be reviewed in details. In terms of disease, retroviruses can be divided into two classes based on how rapidly they cause disease. Acute transforming retroviruses induce tumors rapidly, while non-acute retroviruses induce disease more slowly. Their mechanisms of carcinogenesis are different, and this review will address them separately. Acute transforming retroviruses contain oncogenes which endow viruses with the ability to induce tumors rapidly. However, the common mechanism by which non-acute retroviruses induce cancers is insertional activation of cellular proto-oncogenes. The study of oncogenesis of retroviruses identified several important principles of carcinogenesis including activation of proto-oncogenes and inactivation of tumor suppressor genes that turned out to be important for non-viral cancers of humans as well.

Key words: Retrovirus; Oncogenesis; Oncogene; Insertional activation of proto-oncogenes

1 Introduction

Viruses and cancer have been important subjects for research for over one hundred years. The first reports of cancer viruses in animals were in 1908 for avian myeloblastosis virus (causing an acute myeloid leukemia in chickens)[13] and in 1911 for Rous sarcoma virus (causing fibrosarcoma in chickens)[30]. Beginning in the 1960s, the study of animal cancer viruses identified several important principles of carcinogenesis that turned out to be important for non-viral cancers of humans as well. These include activation of proto-oncogenes and inactivation of tumor suppressor genes. Moreover, it is now estimated that at least 20% of human cancers worldwide have an underlying viral etiology.

In this article oncogenesis by retroviruses will be reviewed. A brief review of retrovirus replication is provided here; more detailed reviews are available elsewhere[5]. Retroviruses are enveloped RNA-containing viruses that have positive stranded RNA (the same sense as viral mRNA) as their genetic material. All retroviruses carry genes encoding viral core proteins (Gag), enzymes (Pol) and envelope (Env) proteins, although various retroviruses also encode additional viral proteins (Fig 1). Infection begins with binding of the virion (the virus particle) to the surface of the infected cell through a specific interaction between viral Env protein and a cellular receptor (Fig 2). Once this interaction takes place the viral core is internalized, followed by activation of virion-bound reverse transcriptase (RT). RT catalyzes synthesis of double-stranded viral DNA which is transported to the nucleus of the cell where viral integrase (IN) integrates it into host's chromosomal DNA. The integrated form of retroviral DNA is referred to as the provirus. The provirus is transcribed by cellular RNA polymerase II, resulting in a viral RNA that is identical to the RNA in virion. This full-length viral RNA is exported to the cytoplasm in unspliced or spliced forms to function as mRNA for Gag and Pol proteins or Env proteins respectively. The initial viral translation products are polyproteins, containing all of the peptide sequences for one viral gene (e.g. $Pr65^{gag}$, the precursor of 65 kDa for the Gag proteins). These polyproteins combine with unspliced viral RNA to form new virions that bud from the cell surface. Once virions are released from the infected cell, the polyproteins are cleaved by virus-encoded protease into the final mature proteins; this results in infectious virus particles.

As a result of the reverse transcription process, the resulting double-stranded viral DNA is somewhat larger than viral genome, containing long terminal

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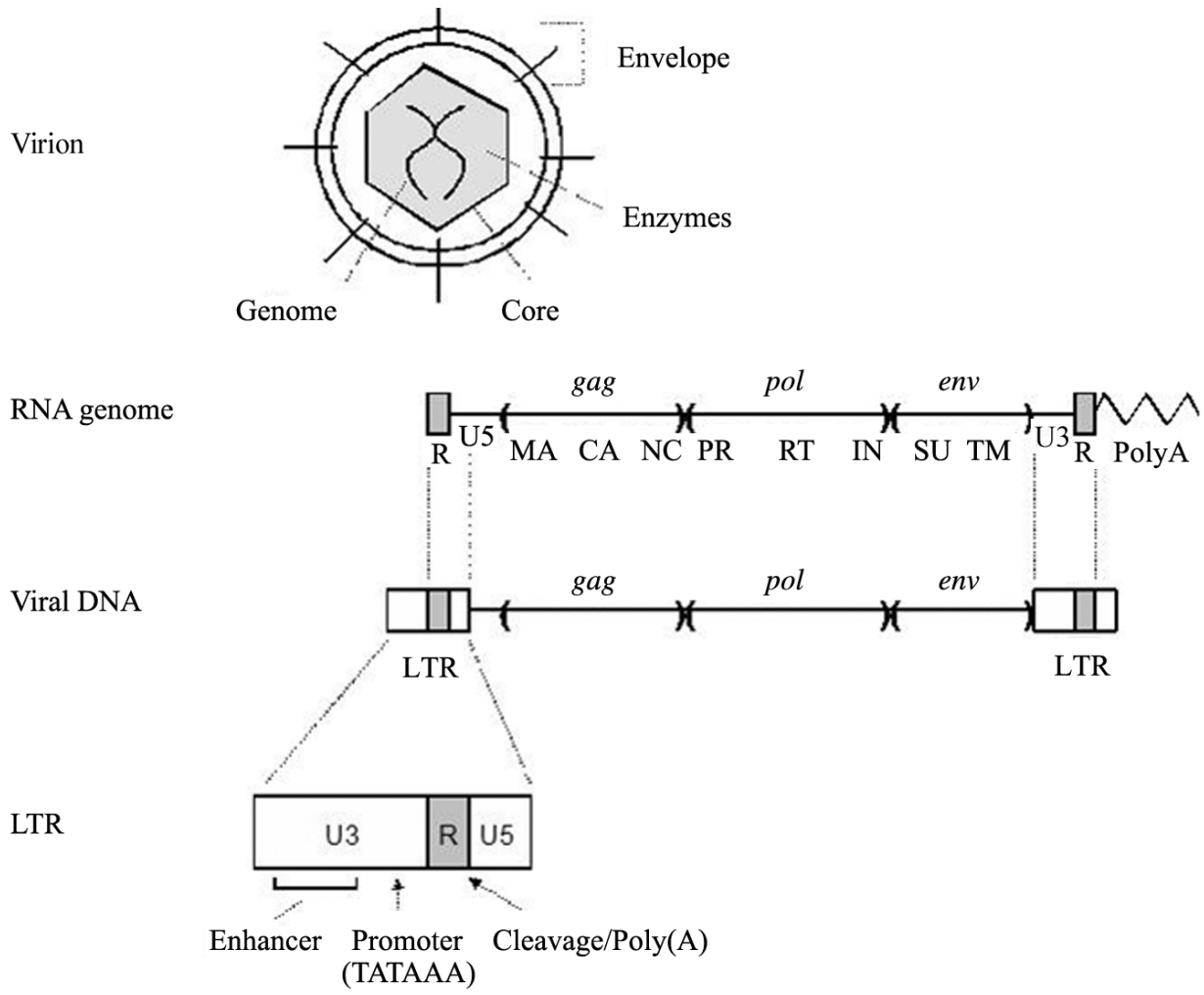

Fig 1. The structure of a retrovirus particle and the organization of the viral RNA genome

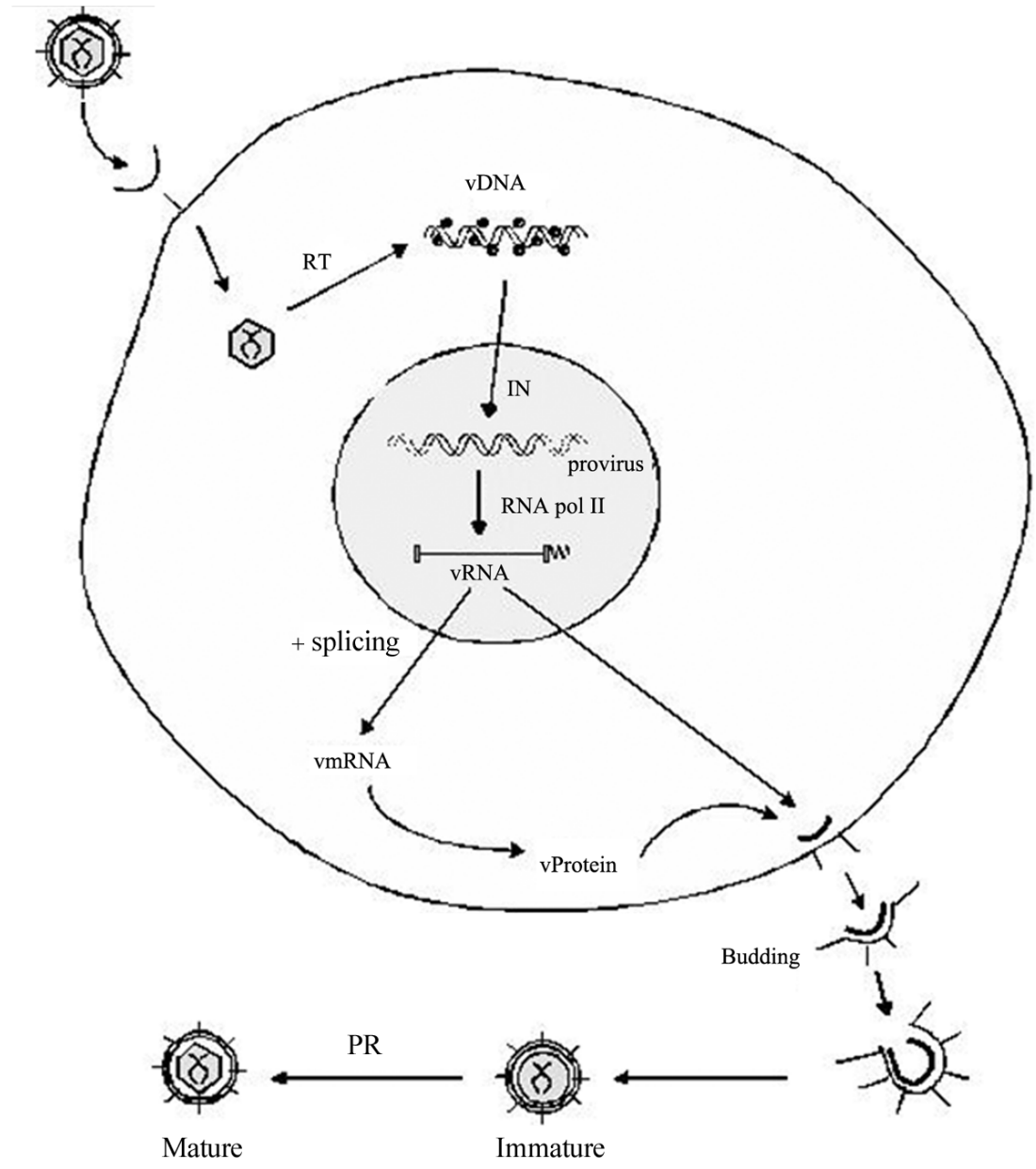

Fig 2. The life cycle of a retrovirus

repeats (LTRs) at either end (Fig 1) . The LTR can be divided into three domains, (U3, R and U5) as also shown in Fig 1. In the infected cell, the integrated provirus closely resembles the original reverse transcribed viral DNA; initiation of RNA synthesis begins at the U3-R junction in the upstream (5 ') LTR, while cleavage/polyadenylation takes place at the R-U5 junction in the downstream (3 ') LTR. The transcriptional control sequences for viral transcription are contained in the enhancer and promoter in the U3 region of the LTR.

In terms of disease, retroviruses can be divided into two classes based on how rapidly they cause disease. Acute transforming retroviruses (e. g. Rous sarcoma virus) induce tumors rapidly (sometimes within days or weeks) , while non-acute retroviruses (e. g. murine leukemia virus) induce disease more slowly (months – years) . Their mechanisms of carcinogenesis are different, and this review will address them separately.

2 Acute transforming retroviruses, oncogenes and induction of disease

Acute transforming retroviruses are characterized by the fact that they contain oncogenes. These genes endow acute transforming retroviruses with the ability to cause rapidly tumors, and in many cases infection of cells in culture with an acute transforming retrovirus changes the growth properties of the cells——transformation. Acute transforming retroviruses are in fact derived from standard (replication-competent) retroviruses, in that the oncogenes have been inserted into their genomes. In many cases this results in an acute transforming retrovirus that is replication-defective. It is dependent on co-infection with a related replication-competent virus (termed a helper virus) in order to infect other cells (Rous sarcoma virus is an exception, in that some strains of RSV are replication-competent, containing all of the retroviral replication genes as well as the oncogene.)

The oncogenes of different acute transforming viruses are different. They originally were named based on the name of the acute transforming retrovirus where they were identified——e. g. the *src* oncogene in RSV. The oncogenes of different acute transforming retroviruses are shown in Tab 1.

Tab 1. Representative acute transforming retroviruses and their oncogenes

Virus	Host Species	Disease	Viral oncogene	Proto-oncogene	Normal proto-oncogene function
Rous sarcoma virus (RSV)	Chicken	Sarcoma	*v-src*	*c-src*	Tyrosine-specific protein kinase
MC29	Chicken	Myeloid leukemia	*v-myc*	*c-myc*	DNA binding protein/Transcription factor
Avian erythroblastosis virus (AEV)	Chicken	Erythroid leukemia	*v-erbA*	*c-erbA*/ thyroid hormone receptor	Hormone-dependent DNA binding protein
			v-erbB	*c-erbB*/ EGFR	Epidermal growth factor receptor tyrosine kinase
E26	Chicken	Erythroid leukemia	*v-ets*	*Ets-1*	DNA binding protein/Transcription factor
Avian myeloblastosis virus (AMV)	Chicken	Myeloid leukemia	*v-myb*	*c-myb*	DNA Binding protein/transcription factor
Reticuloendotheliosis virus-T (REV-T)	Turkey	Reticuloendo-theliosis	*v-rel*	*c-rel*	NF KappaB subunit
S17 sarcoma virus	Chicken	Sarcoma	*v-jun*	*c-jun*	DNA binding protein/AP-1 transcription factor
Harvey murine sarcoma virus	Mouse	Sarcoma	$v\text{-}ras^{H}$	*H-ras*	G-protein involved in signal transduction
Kirsten murine sarcoma virus	Mouse	Sarcoma	$v\text{-}ras^{K}$	*K-ras*	
Murine sarcoma virus 3611	Mouse	Sarcoma	*v-raf*	*c-raf*	Serine/threonine protein kinase involved in signal transduction (MEKK)
Abelson murine leukemia virus	Mouse	B-lymphoma	*v-abl*	*c-abl*	Tyrosine protein kinase
AKT-8 murine leukemia virus	Mouse	T-lymphoma	*v-Akt*	*Akt*	Serine/threonine protein kinase in the PI3K signaling pathway
FBJ osteosarcoma virus	Mouse	Osteosarcoma	*v-fos*	*c-fos*	Component of AP-1 transcription factor (with c-Jun)
Feline sarcoma virus (McDonough strain)	Cat	Sarcoma	*v-fms*	*c-fms*/ CSF-1R	Receptor tyrosine kinase; receptor for CSF-1

Depending on the acute transforming retrovirus, the nature of the oncogene protein varies. For some, the viral oncogene is expressed as a distinct protein from its own mRNA (e. g. the Src protein of RSV) [2]. For others the oncogene protein is expressed as a fusion with viral protein (e. g. the Gag-abl fusion oncogene of Abelson MuLV) [38].

A major (Nobel prize-winning) discovery about the nature of oncogenes of acute transforming retroviruses was that they were derived from normal cell genes[34]. These normal cell genes are called proto-oncogenes. The nomenclature is that an oncogene present in an acute transforming retrovirus is called a viral oncogene (e. g. *v-src* for RSV) , while its normal cell counterpart is the cellular proto-oncogene (e. g. *c-src* corresponding to *v-src*) . As a group, proto-oncogenes are involved in positive stimulation of cell division or response to mitogenic signals. During the 1980s retroviral oncogenes and proto-oncogenes played major roles in elucidating pathways of cellular signaling and growth control. Such cellular proteins such as Ras, Myc, Myb, Abl, Raf, Fos, Jun and ErbB (EGFR) were all discovered originally as cellular counterparts of viral oncogenes. In addition, study of viral oncogenes identified the biochemical mechanisms of action for many proto-oncognes. Most notably, the tyrosine kinase activities of Src[7,18], Abl[37], EGFR and growth factor receptor proteins[6] were discovered. The GTPase activities of the Ras proteins and the DNA binding activities of Jun and Fos proteins were all identified by the study of viral oncogenes or cellular proto-oncogenes.

A common feature of viral oncogene proteins compared to cellular proto-oncogene proteins is that they show dysregulated function. That is the cellular proto-oncogene proteins stimulate signaling or cell division in a limited (controlled) fashion, while viral oncogene proteins stimulate division in an uncontrolled constitutive fashion. For instance the v-Src protein has a mutated C-terminus compared to the cellular c-Src protein. This results in the loss of a C-terminal tyrosine residue that is the target for a regulatory phosphorylation in the cellular c-Src protein. As a result v-Src protein has constitutive tyrosine kinase activity.

3 Non-acute retroviruses and activation of proto-oncogenes

Non-acute retroviruses are typical replication-competent retroviruses. In many cases they induce cancers, predominantly leukemias of different kinds, but for a few viruses carcinomas. The rate at which non-acute retroviruses induce cancers is much slower than for non-acute retroviruses, and infected animals develop high levels of viral infection well in advance of appearance of tumors.

The common mechanism by which non-acute retroviruses induce cancers is insertional activation of cellular proto-oncogenes. This was first identified in studies of B-cell leukemias in chickens induced by avian leukosis virus (ALV) [16]. While retroviruses integrate their viral DNA into the host chromosomes at multiple sites (almost random for ALV) , the laboratories of Hayward and Astrin observed that multiple independent ALV-induced tumors showed insertion at the same chromosomal site, and they identified this site as the chicken *c-myc* locus. This integration resulted in transcriptional readthrough from the viral LTR into the *c-myc* gene, resulting in a highly expressed fusion transcript under control of the highly active ALV LTR. As a result the c-Myc protein is over-expressed constitutively, leading to a signal for uncontrolled growth. Hayward *et al.* termed this mechanism promoter insertion (Fig 3) .

After the initial discovery of *c-myc* activation in ALV-induced tumors, other investigators found that this proto-oncogene activation is common to tumors induced by non-acute retroviruses. Depending on the particular virus, different proto-oncogenes are activated. For instance when Moloney murine leukemia virus (M-MuLV) induces T-lymphomas, it activates *c-myc*[33], another proto-oncogene *pim-1*[8] or others. Furthermore, many non-acute retroviruses use a variant mechanism of proto-oncogene activation (Fig 3) . For these viruses, transcriptional activation of the proto-oncogene ’s own promoter occurs by integration of the highly active enhancer sequences in the viral LTR in the vicinity of the proto-oncogene. For these tumors the provirus is often inserted in the opposite transcriptional orientation from the proto-oncogene, or downstream of

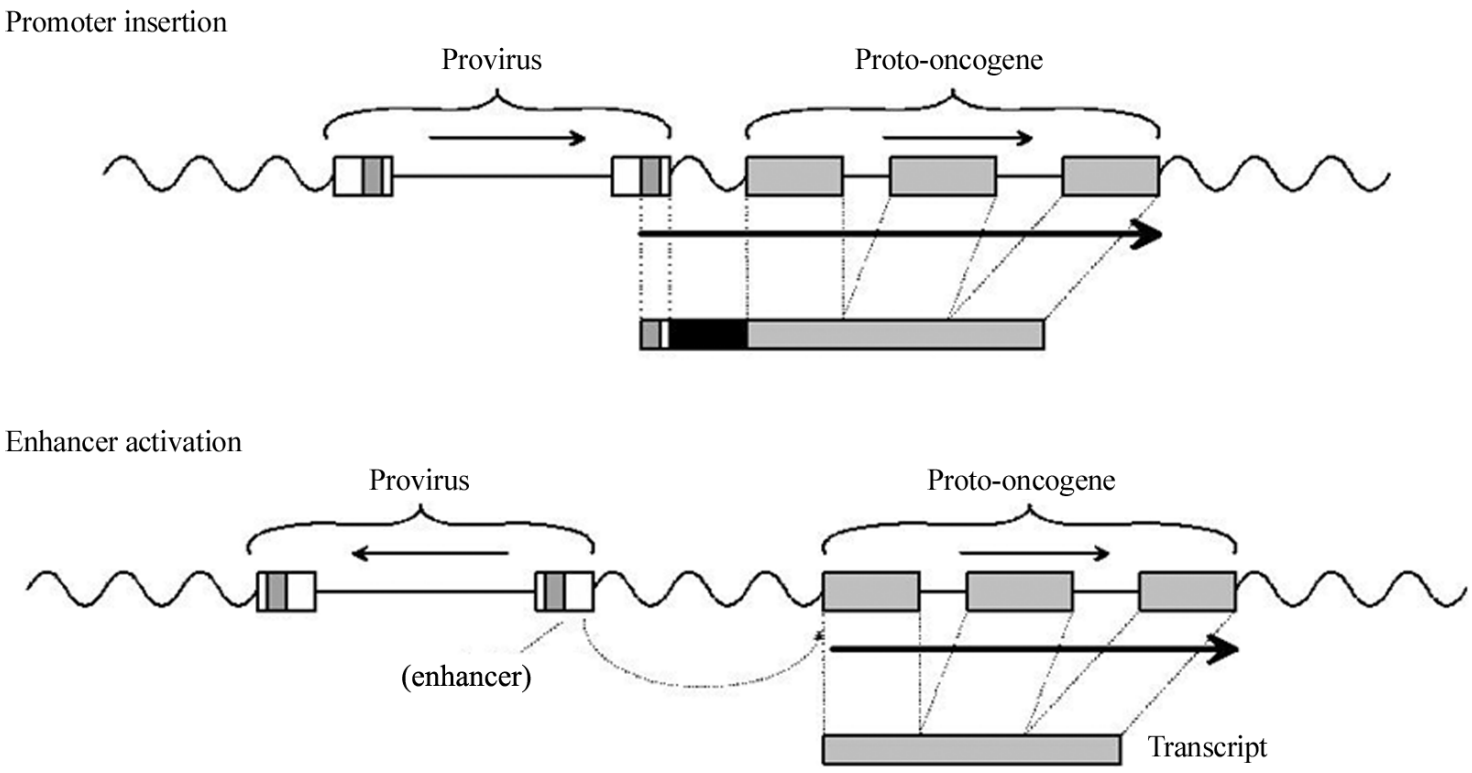

Fig 3. Mechanism of activation of proto-oncogen

it. This is explained by the fact that enhancer sequences can work in orientation and direction-independent fashions relative to a promoter.

In sum non-acute retroviruses induce tumors by a general mechanism that can be termed LTR activation of proto-oncogenes. There are two sub-mechanisms, promoter insertion and enhancer activation. This mechanism also explains why non-acute retroviruses induce disease slowly in comparison to acute transforming retroviruses. Since retroviral integration can occur in multiple chromosomal loci (almost random for some viruses) , the likelihood of insertion next to a particular proto-oncogene in any given cell is low. Multiple rounds of infection (and time) are required before insertion occurs next to a critical proto-oncogene in one cell in the infected organism. It is that cell that will then develop into the tumor.

Non-acute retroviruses have also been used in discovery of new cellular proto-oncogenes. Researchers search collections of tumors induced by a particular retrovirus for common regions of proviral insertion. These common insertion sites are indicative of activation of a proto-oncogene. The proto-oncogenes are then identified as cellular genes in the common insertion sites that show over-expression in the tumors. For example study of mammary tumors induced by mouse mammary tumor virus (MMTV) identified proto-oncogenes, initially named *int-1*, *int-2* and *int-3*[27]. These are now referred to as *wnt* genes. The Wnt signaling pathway was subsequently found to be a major regulatory pathway for normal cell development and abnormalities of cancer. The proto-oncogene *pim-1* (see above) was also discovered as a common insertion site in M-MuLV-induced T-lymphomas[8]. Discovery of cellular proto-oncogenes has recently been accelerated by employing genomic techniques (high throughput DNA sequencing) in analysis ot tumors induced by non-acute retroviruses[21]. These genomic techniques have allowed rapid identification of common proviral insertion sites in tumors, which has substantially accelerated the discovery of new proto-oncogenes.

Different non-acute retroviruses induce specific kinds of tumors. It has been found that the major determinant for the disease specificity is the LTR. For instance Moloney MuLV induces T-lymphoma while Friend MuLV induces erythroleukemia in mice. If just the LTRs are exchanged by molecular cloning, then the type of disease sorts with the LTR. In fact just insertion of F-MuLV enhancers (from the U3 region of the LTR) into M-MuLV results in a virus that causes erythroleukemia, and *vice versa*[22]. This can be explained by the fact that enhancers (both viral and cellular) show cell-type specificity, because they bind transcription factors that are present in particular types of cells. Thus a retrovirus whose enhancers are active in a particular differentiated cell type will efficiently

activate proto-oncogenes in that cell type, resulting in tumors.

4 Activation of proto-oncogenes in non-viral tumors

The discovery of cellular proto-oncogenes through studies of retroviruses became even more important when it was found that proto-oncogenes are also activated in naturally occurring human tumors. By and large these tumors do not result from retroviral infection. There are several different mechanisms that lead to activation of proto-oncogenes. First there are tumors over-express normal proto-oncogene proteins. This can occur by two mechanisms. First, in some tumors there is amplification of a region of the chromosome containing a proto-oncogene——sometimes evident as a homogeneously staining region, or as micro-satellites. For example, the *her*-2 /*neu* proto-oncogene is amplified in a subset of breast and ovarian cancers[31]. Indeed tumors with *her*-2 /*neu* amplification have a worse clinical prognosis, which has led to typing of breast and ovarian cancers for this amplification as part of tumor staging. *her*-2 /*neu* is the target of a specific drug (Herceptin, a monoclonal antibody) that is useful in treatment of tumors showing this amplification[32]. The second mechanism by which normal proto-oncogene proteins can be over-expressed is through chromosomal translocation. Characteristic translocations are present in some kinds of tumors, and this often involves translocation of a proto-oncogene. In Burkitt's lymphoma there is a characteristic 8 :21 chromosomal translocation. This results in movement of the *c-myc* proto-oncogene next to the structural gene for the heavy chain of immunoglobulin[9]. Since Burkitt's lymphoma is a tumor of B-lymphocytes, this result in translocation of *c-myc* next to a gene that is highly expressed, resulting in over-expression of c-Myc as well.

The second mechanism of proto-oncogene activation in human tumors involves alteration of the proto-oncogene protein itself. For example the *ras* proto-oncogenes are activated in tumors by single base mutations. The Ras proteins are small G-proteins involved in signal transduction; they are cyclically activated and inactivated by binding of GTP or GDP. Tumor mutations lead to single amino acid substitutions in the Ras proteins resulting in loss of intrinsic GTPase activity[26]. These mutant Ras proteins are unable to hydrolyze bound GTP to GDP and they are constitutionally locked into an active configuration which leads to constitutive signaling for cell growth. The second mechanism by which proto-oncogene protein activities are altered is through chromosomal translocation. For example the Philadelphia chromosome is a translocation characteristic of chronic myelogenous leukemia (CML) and certain B-cell leukemias. In this case the translocation leads to a new fusion transcript across the translocation, containing bcr sequences from one chromosome fused to sequences of the *c-abl* proto-oncogene from the other chromosome[11, 15]. This in turn results in a Bcr-Abl fusion protein, which has increased tyrosine-specific protein kinase activity compared to c-Abl. Recently a small molecule inhibitor of Bcr-Abl protein kinase has been developed (Imatinib) that has become the frontline therapy for treatment of CML.

5 Micro-RNAs

RNA interference (RNAi) is a recently discovered mechanism of gene expression control. In particular, small double-stranded micro-RNAs have been recently discovered[3]. These RNAs result from processing of larger cellular transcripts, and the micro-RNAs down-regulate expression of other cellular mRNAs by RNAi mechanisms. It is a newly discovered mechanism of cellular gene regulation, and by and large it modulates the levels of activity of their target genes. The first discovery of a micro-RNA actually occurred through study of B-lymphoid tumors induced in young chicks by an avian non-acute retrovirus[4]. These tumors showed proviral DNA integration near a common insertion site that was given the name *bic*. The insertions led to over-expression of *bic* transcripts, which suggested that it was a proto-oncogene. However, the *bic* RNA did not have any open reading frames that could encode protein, so its mechanism of action was mysterious at the time[35]. After the discovery of RNAi and micro-RNAs, it was determined that *bic* actually encodes a micro-RNA termed mir155[20]. mir155 is important in normal lymphoid

development, so over-expression of this micro-RNA would logically lead to lymphoma.

6 Other mechanisms of retroviral oncogenesis

While the predominant mechanisms of retroviral oncogenesis have been described above, a small number of oncogenic retroviruses employ distinct mechanisms.

6.1 Human T-cell leukemia virus (HTLV) and bovine lymphosarcoma virus (BLV)

These two retroviruses belong to the deltaretrovirus family and they cause adult T-cell leukemia (ALT) and B-lymphoma. There are two major strains of HTLV, HTLV-I and -II; only HTLV-I is associated with disease. There are approximately 20 million people infected with HTLV-I worldwide, with high regions of infection including Japan, the Caribbean, Latin America, and Africa. In these regions, ATL is more common, due to the relatively high incidence of infection. HTLV-I also induces a neurological disease, HTLV-associated myelopathy (HAM), also called tropical spastic paraparesis (TSP).

HTLV-I induces ATL in infected individuals relatively inefficiently and with a long latency (typically more than 20 years) —— the rate of disease development is 1%-2% per year. Both HTLV and BLV carry additional genetic information compared to typical retroviruses, the X region. This region is expressed into several proteins by way of alternately spliced mRNAs, including Tax (a transcriptional transactivator) and Rex (a protein that facilitates cytoplasmic of unspliced viral RNA). Tax transactivates the HTLV LTR, but also the promoters of several cellular genes, including interleukin-2 (IL-2) and IL-2 receptor[39]. HTLV-I Tax may function as an oncogene since it can transform rodent fibroblasts in culture, and it is necessary for the ability of HTLV-I to immortalize primary human T-lymphocytes. Immortalization of T-lymphocytes may result from Tax-induced expression of both IL-2 and IL-2 receptor in the same cell, resulting in an autocrine loop of IL-2-driven division. Thus Tax may allow HTLV-I to establish a pre-leukemic state in infected T-lymphocytes which persists for many years. Paradoxically, when ATL tumors develop, the cells no longer express Tax, even though they contain the HTLV-I provirus. There appears to be selection against continued Tax expression during the development of the tumors.

On the other hand, another HTLV-I protein is expressed in ATL tumors, HBZ[14]. This protein is a B-zip DNA binding protein; it is encoded by an mRNA transcript originating from the opposite strand of the viral DNA, initiated in the downstream LTR. The fact that HBZ is expressed in all ATL tumors suggests that it may be important for development or replication of ATL cells. Thus two HTLV-I proteins may be involved in development of ATL: Tax may be involved in initiation (but not maintenance) of the tumorigenic process while HBZ may be involved in maintenance of oncogenic transformation.

6.2 Jaagsiekte sheep retrovirus (JSRV)

JSRV is a member of the betaretrovirus family, and it causes a transmissible lung cancer in sheep, ovine pulmonary adenocarcinoma (OPA). OPA is a tumor of secretory epithelial cells of the alveoli, type II pneumocytes, and Clara cells of the bronchioles. The normal function of type II pneumocytes is production of pulmonary surfactant that maintains the gas permeability of the alveoli. Animals in end-stage disease are in respiratory distress because the tumor cells secrete excess surfactant. "Lung fluid" can be recovered from these animals, and the lung fluid contains infectious virus. It is believed that spread of the viral infection and cancer is by aerosol droplets of lung fluid that are then inhaled by a recipient animal.

When JSRV was molecularly cloned, sequencing of the genome revealed standard retroviral genes (*gag*, *pol* and *env*) with no indication of a transduced viral oncogene (i.e. sequences with homology to some cellular gene). This suggested that JSRV might induce tumors by LTR activation of proto-oncogenes. However, a tumor cell line from a JSRV-induced tumor containing only one copy of proviral DNA was found to contain the viral DNA integrated into the structural gene for pulmonary surfactant A—— which is not likely to be a proto-oncogene[12]. This argued against LTR activation of proto-oncogenes as the mechanism of JSRV oncogenesis. At the same time, JSRV is a potent carcinogen: intratracheal inoculation of virus into

newborn lambs results in OPA with a mean latency of 6 weeks, with some animals developing disease in as little as 2 weeks. This would be more consistent with JSRV carrying an oncogene, even though it was not evident from the viral DNA sequence. Indeed when JSRV DNA was transfected into rodent fibroblasts (e.g. NIH-3T3 or rat 208F)[25] or epithelial cell lines (e.g. rat RK3E cells)[24], foci of transformed cells developed, and the transformed cells were tumorigenic. The most unusual feature was that the *env* gene of JSRV was sufficient to cause transformation. Thus JSRV Env protein functions as an oncogene for the virus. Indeed, introduction of JSRV protein alone into the lungs of SCID mice by way of an adeno-associated virus vector is sufficient to cause lung cancer.

Retroviral envelope proteins are initially translated as a polyprotein precursor containing sequences for the two envelope proteins Surface (SU) and transmembrane (TM), with SU at the amino terminus and TM at the C-terminus. The SU protein is on the outside of the virus particle (it contains the receptor binding domain) while the TM protein spans the lipid bilayer of the virus particle. TM anchors SU to the virus particle. It is presumed that in JSRV-transformed (or tumor) cells the same organization is present——the SU protein is on the surface of the cell while the TM protein spans the plasma membrane with a C-terminal tail exposed to the cytoplasm. Structure-function analysis of the Env protein indicated that sequences in the short 45 amino acid cytoplasmic tail of TM are necessary for transformation[28]. They presumably interact with cellular proteins, leading to increased signals for growth. Two signaling pathways have been shown to be important for JSRV Env transformation: the PI3K-AKT-mTOR pathway, and the Ras-MEK-ERK pathway[24]. Identification of the cellular proteins involved in JSRV transformation is currently under way.

Other parts of the JSRV Env protein may also be important for oncogenesis. The SU protein binds to the receptor, which is hyaluronidase-2(Hyal-2)[29]. Both human and sheep Hyal-2 function as receptors for JSRV, although rodent Hyal-2 does not. It is interesting that Hyal-2 maps to human chromosome 3p21.3, which also is a region of common deletion in lung cancers. Chromosomal deletions in cancers are often associated with the loss of tumor suppressor genes, so this led to the idea that JSRV Env may also interfere with a tumor suppressor function of Hyal-2. Evidence supporting this model has been reported. In a human lung epithelial cell line, the Hyal-2 protein is found in a complex with a membrane-spanning receptor-type tyrosine kinase, Stk (also known as RON)[10]. The kinase activity of Stk/RON is inhibited when it is complexed with Hyal-2. Expression of JSRV Env in these cells results in binding of Hyal-2 to JSRV SU, releasing Stk/RON from the inhibitory complex. Thus SU may be involved in JSRV tumorigenesis in sheep by binding Hyal-2 and increasing signaling through Stk/RON. However when JSRV Env induces tumors in rodents or transforms rodent cells, this mechanism does not contribute to the transformation/tumorigenesis since JSRV Env does not bind to rodent Hyal-2.

6.3 MMTV

MMTV is also a betaretrovirus that induces mammary tumors and B-lymphomas. When MMTV induces these tumors it employs insertional activation of proto-oncogenes. However recently it has been found that the Env protein may also contribute to tumorigenesis. When a murine mammary epithelial line is infected with MMTV, it shows no changes when grown on plastic. However, if these cells are grown in 3D culture, suspended in medium containing extracellular matrix components (Matrigel), the uninfected cells form small spheres of polarized epithelial cells[19]. However, the infected cells show abnormalities when grown in Matrigel, with formation of larger spheres and lack of polarization. The ability of MMTV to induce these changes was traced to the Env protein. In particular an ITAM motif in the MMTV Env protein was found to be responsible.

7 Viruses associated with human cancer

As mentioned in the introduction, approximately 20% of human cancers are caused by viral infection. Tab 2 shows the currently known viruses and the cancers that they are associated with. They include DNA viruses and RNA viruses. In some cases they are

direct-acting carcinogens (i. e. expression of the virus or a viral gene product in the tumor cell is driving oncogenesis) , while in other cases they may cause cancer indirectly (the virus is not in the tumor cell) . The oncogenic viruses that are not retroviruses will not be reviewed here.

Tab 2. Viruses associated with human cancer

Virus classification	Virus	Cancer
Herpesvirus	Epstein-Barr virus (EBV)	Burkitt ’s lymphoma
		AIDS lymphomas
		Hodgkin ’s disease
		Naso-pharyngeal carcinoma
Herpesvirus	Kaposi ’s sarcoma herpes virus (KSHV or HHV-8)	Kaposi ’s sarcoma
		Pleural effusion lymphoma
		Multi-centric Castleman ’s disease
Papilloma virus	Human papillomavirus (HPV types 16, 18, 31, 35)	Cervical cancer
		Other cancers of the reproductive tract (penis, vulva)
		Cancer of the tonsils
Polyoma virus	Merkel cell carcinoma virus (MCV)	Merkel cell carcinoma
Hepadnavirus	Hepatitis B virus	Hepatocellular carcinoma
Flavivirus	Hepatitis C virus	Hepatocellular carcinoma
Retrovirus	Human T-cell leukemia virus type 1 (HTLV-1)	Adult T-cell leukemia
Retrovirus	Human immunodeficiency virus (HIV)	AIDS malignancies
		Kaposi ’s sarcoma, lymphomas, cervical cancer
Retrovirus	Xenotropic MulV-related retrovirus (XMRV)	Prostate cancer?

7. 1 HTLV-I

HTLV-I and its role in inducing ATL is described in Section 6. 1.

7. 2 Human immunodeficiency virus type 1 (HIV-1)

HIV-1 is the causative agent of AIDS. As the immune system becomes defective in AIDS patients, cancers develop. Thus HIV-1 can be considered to induce cancers indirectly. AIDS-associated cancers include Kaposi ’s sarcoma, AIDS lymphomas and cervical cancer. It is interesting that the AIDS-associated cancers all result from infection by another oncogenic virus. Thus the immunodeficiency of AIDS may be resulting in cancers principally by reducing resistance to infection by the oncogenic viruses. Indeed, cancers that are not associated with viral infections are not typically elevated in AIDS patients (e. g. lung cancer) .

7. 3 xenotropic MuLV-related virus (XMRV)

Recently a new human retrovirus has been identified, XMRV. This virus was detected in patients with a familial form of prostate cancer, in whom a component of innate immunity is defective[36] . RNase L is an effector protein in the interferon antiviral response mechanism, and individuals who are homozygous for the R462Q mutation show a two-fold elevated risk for development of prostate cancer. This led to the hypothesis that these individuals are more susceptible to infection by a virus that causes prostate cancer, and 40% of tumors from these cases showed evidence for infection with a novel retrovirus, XMRV. It is interesting that XMRV is closely related to MuLV, which suggests that it might represent cross-species infection of humans with a murine retrovirus. Experiments on XMRV are still in their infancy, and the role of this virus in human prostate cancer (and the mechanism of oncogenesis) still remains to be determined.

7. 4 Endogenous retrovirus (ERV)

One of the features of the retrovirus life cycle is the integration of viral DNA into the host chromosome. If retroviral infection takes place in a germ cell that goes on to form an embryo, then any progeny will transmit the retroviral provirus to their offspring as standard genetic elements. These inherited proviruses are referred to as ERVs. During evolution this process has occurred frequently; in fact approximately 8% of the total human genome consists of human endogenous

retroviruses (HERVs) . Most of these HERVs are replication-defective, which probably represents selection against replication-competent HERVs. However, many HERVs can encode functional proteins, if not whole viruses, and it is possible that HERV proteins can participate in tumorigenesis. Certain kinds of human tumors have been found to express HERVs (e. g. seminomas)[17], which supports this possibility. Also, the HERV-K encodes a small viral regulatory protein[23] that can confer tumorigenicity to rodent fibroblasts[1].

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反转录病毒感染与肿瘤发生

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摘 要: 病毒感染与癌症的关系是生物医学领域中非常重要的研究方向, 估计当前全世界至少 20% 人类肿瘤的发生与病毒感染有密切联系。本文对反转录病毒诱发肿瘤的各种作用机制进行了详细阐述。根据致病速度的快慢, 反转录病毒被分为两大类: 能迅速诱导肿瘤产生的急性转化反转录病毒和缓慢诱导肿瘤产生的非急性转化反转录病毒。急性转化反转录病毒通过其自身携带的癌基因快速诱导肿瘤产生, 而细胞原癌基因的插入激活则是非急性转化反转录病毒引起肿瘤的主要机制。对反转录病毒致瘤机制的研究揭示了肿瘤发生过程中的一些重要原理和机制, 包括原癌基因激活以及肿瘤抑制基因失活等, 对探讨非病毒引起的人类肿瘤的发生机制同样具有重要的参考意义。

关键词: 反转录病毒; 肿瘤发生; 癌基因; 原癌基因插入激活

1 前言

病毒感染与癌症的关系是 100 多年来生物医学领域中非常重要的研究方向。早在 1908 年科学家就发现禽类成髓性白血病病毒(avian myeloblastosis virus, AMV) 能感染鸡并引发急性髓系白血病(acute myeloid leukemia, AML)[13]。1911 年又发现劳斯肉瘤病毒(Rous sarcoma virus, RSV) 能诱发鸡纤维肉瘤(fibrosarcoma)[30]。这些是最早有关病毒引发动物肿瘤的报道。从 20 世纪 60 年代开始, 对动物肿瘤病毒的研究揭示了病毒引发动物肿瘤发生的重要原理和机制, 如原癌基因(proto-oncogene) 的激活以及肿瘤抑制基因(tumor suppressor gene) 的失活等, 这些理论对于探索和理解非病毒引起人类肿瘤的发生机制同样具有重要的参考意义。据估计, 当前全世界至少 20% 人类肿瘤的发生与病毒感染有密切联系。

本文对反转录病毒(retrovirus) 的致瘤机制作一综述。首先, 简单介绍反转录病毒的复制过程(更具体详细的阐述可参考相关的综述文章)[5]。反转录病毒是具有包膜的正链 RNA 病毒, 自身可编码病毒核心蛋白(Gag)、聚合酶(Pol) 以及包膜蛋白(Env), 不同种属的反转录病毒还额外编码一些其他病毒蛋白(图 1)。病毒感染起始于病毒颗粒与被感染细胞的结合, 病毒的包膜蛋白通过与相应的细胞表面受体发生特异性的相互作用, 介导病毒感染(图 2)。随后, 脱去包膜的病毒核心颗粒穿入细胞, 包裹在核心颗粒中的反转录酶(reverse transcriptase, RT) 被激活, 催化合成双链病毒 DNA。新生成的双链病毒 DNA 被转运入细胞核并在病毒整合酶(integrase, IN) 的作用下插入宿主细胞的染色体 DNA, 这种整合形式的病毒 DNA 被称为前病毒(provirus)。前病毒在宿主细胞的 RNA 聚合酶Ⅱ的作用下转录出与包含在病毒颗粒中的基因组 RNA 完全一致的病毒 RNA。这种完整的病毒 RNA 转运至胞质后被剪切成病毒 mRNA, 以此为模板分别合成病毒的核心蛋白、聚合酶以及包膜蛋白。最初的病毒翻译产物是一些聚合蛋白前体, 携带了由 1 个病毒基因编码的全部肽链序列(如 $Pr65^{gag}$ 是 65 000的 Gag 蛋白前体)。这些聚合蛋白前体与未被剪切的病毒基因组 RNA 结合, 形成子代病毒颗粒并以出芽的方式从细胞表面释放。在病毒颗粒被感染细胞释放的过程中, 病毒编码的蛋白酶会将聚合蛋白前体切割成最终的成熟蛋白, 从而产生具有感染力的成熟病毒颗粒。

经反转录产生的双链病毒 DNA, 两端含有长末端重复序列(long terminal repeat, LTR) (图 1), 比病毒基因组稍大。LTR 具有 3 个结构域, 分别为 U3、R 和 U5。整合于被感染细胞中的前病毒非常类似于经反转录产生的病毒 DNA。病毒 RNA 的合成起始于上游(5 端) LTR 的 U3-R 连接区域, 并在下游(3′端) LTR 的 R-U5 连接区域发生 mRNA 的切割以及聚合腺 哌化而转录终止。病毒转录的调控序列主要集中在 LTR U3 结构域中的增强子和启动子内。

根据致病速度的快慢,反转录病毒被分为两大类:能迅速诱导肿瘤产生(通常只需几天或几周时间)的急性转化反转录病毒(acute transforming retrovirus),如 RSV;以及缓慢诱导肿瘤产生(几个月甚至几年)的非急性转化反转录病毒(non-acute retrovirus),如小鼠白血病病毒(murine leukemia virus, MuLV)。这两类病毒引发肿瘤的机制是完全不同的,本文将分别阐述。

2 急性转化反转录病毒、癌基因以及肿瘤发生机制

急性转化反转录病毒的最主要特征是具有癌基因(oncogene),正是这些癌基因赋予其快速诱导肿瘤产生的能力。在很多体外培养系统里,感染了急性转化反转录病毒的细胞,生长增殖的特性发生了变化,最终导致细胞发生了转化。急性转化反转录病毒来源于具有正常复制能力的普通反转录病毒,但其基因组中插有癌基因。在绝大多数情况下,急性转化反转录病毒是复制缺陷型的,病毒复制必须依赖于相应的可复制型病毒(称为辅助病毒,helper virus)的共感染。RSV 则例外,因为其某些毒株是可复制型的,包含所有的病毒复制相关基因以及癌基因。

不同的急性转化反转录病毒携带不同的癌基因。这些癌基因通常根据其所在的病毒而命名,如在 RSV 中发现的癌基因被命名为 *src*。表 1 列出了不同急性转化反转录病毒中含有的癌基因。

癌基因的特性根据其所处病毒的不同而有差异。有些病毒癌基因蛋白是作为 1 个单独的蛋白表达的,如 RSV 中的癌基因蛋白 Src[2];而其他的癌基因蛋白则与病毒蛋白发生融合而共表达,如 Abelson MuLV 中的 Gag-Abl 融合癌基因蛋白[38]。

研究表明,急性转化反转录病毒中的癌基因来自于被称为原癌基因的正常细胞基因[34]。为区分两者,位于病毒中的癌基因被称为病毒癌基因,如 RSV 中 *v-src*,而细胞中相对应的正常基因则被称为细胞原癌基因,如对应于 *v-src* 的细胞原癌基因 *c-src*。细胞原癌基因能促进细胞分裂并参与有丝分裂信号的应答。在 20 世纪 80 年代,对反转录病毒癌基因以及细胞原癌基因的研究为阐明细胞信号通路以及生长控制过程起了非常重要的作用。包括 Ras、Myc、Myb、Abl、Raf、Fos、Jun 以及 ErbB〔表皮生长因子受体(epidermal growth factor receptor, EGFR)〕在内的诸多重要细胞蛋白最初都是作为病毒癌基因在细胞内的对应物而被发现的。此外,对病毒癌基因的研究同时也阐明了许多细胞原癌基因的生化作用机制。最著名的例子就是 Src[7,18]、Abl[37]、EGFR 以及生长因子受体(growth factor receptor, GFR)[6]的酪氨酸激酶活性的发现。此外,Ras 蛋白的 GTPase 活性以及 Jun 与 Fos 蛋白的 DNA 结合能力也是通过对病毒癌基因与细胞原癌基因的研究而揭示的。

有别于细胞原癌基因蛋白,病毒癌基因蛋白的共同特性在于其功能调控是失常的。细胞原癌基因蛋白以一种受控的方式刺激信号产生,调控细胞分裂,而病毒癌基因蛋白则以不受控制的方式持续刺激细胞分裂。如与 c-Src 蛋白相比,v-Src 蛋白的 C 端发生了突变,导致具有磷酸化调节功能的酪氨酸位点发生丢失,以至于 v-Src 蛋白具有持续性的酪氨酸激酶活性。

3 非急性转化反转录病毒与原癌基因的活化

非急性转化反转录病毒是一类典型的具有复制能力的反转录病毒,能诱导癌症发生,主要为各种不同类型的白血病,也有一小部分病毒能诱发恶性实体瘤。非急性转化反转录病毒诱导肿瘤产生的速度比急性转化型病毒要漫长得多,而且被感染的个体在肿瘤出现前会经历高水平的病毒感染状态。

细胞原癌基因的插入激活是非急性转化反转录病毒引发肿瘤的主要机制,是在研究由禽类白血病病毒(avian leukosis virus, ALV)引发的鸡 B 细胞白血病中首先发现的[16]。Hayward 以及 Astrin 实验室的研究发现,反转录病毒能将病毒 DNA 整合入宿主细胞染色体的多个位点,而且对于 ALV 来说,整合位点通常是随机的,但在许多独立形成的肿瘤中 ALV 的插入位点却总是相同的,这一整合位点被命名为 *c-myc* 基因座。ALV 的插入导致细胞 *c-myc* 基因从病毒 LTR 区域开始发生转录通读,使得 *c-myc* 与病毒的融合蛋白在具有高转录活性的 LTR 控制下大量转录、表达。过量持续表达的 c-Myc 蛋白刺激产生不受控制的细胞生长信号。Hayward 等将这一机制称为启动子插入激活(图 3)。

自发现 *c-myc* 基因的插入激活是 ALV 诱导肿瘤产生的机制以来,许多学者相继发现细胞原癌基因激活是非急性转化反转录病毒诱发肿瘤的一个常见机制。根据感染病毒种类的不同,机体被活化的细胞原癌基因也相应不同。如在 Moloney 小鼠白血

病病毒(Moloney murine leukemia virus, M-MuLV) 诱发的 T 细胞淋巴瘤中,病毒能将 *c-myc*[33]、*pim-1*[8] 或其他细胞原癌基因激活。此外,很多非急性转化反转录病毒还利用另外一种机制激活原癌基因并诱导肿瘤发生(图 3) 。这些病毒 LTR 区域中的高活性增强子序列被插入原癌基因附近,从而增强原癌基因自身启动子的转录活性。在这类肿瘤细胞中,前病毒经常以与原癌基因转录相反的方向插入,或者被插入在原癌基因的下游。增强子序列以启动子方位依赖型的方式发挥作用。

总之,LTR 激活原癌基因是非急性转化反转录病毒诱导肿瘤发生的主要机制,包括启动子插入激活和增强子激活。这一机制也解释了与急性转化型病毒相比,非急性转化反转录病毒诱导肿瘤发生的速度相对较慢的原因。由于反转录病毒的整合能发生于细胞染色体的多个位点(对于有些病毒来说几乎是随机插入) ,病毒在细胞特定原癌基因附近插入的概率很低。因此需要反复多次以及长时间的感染才能保证病毒插入关键的细胞原癌基因中,随后该细胞才能逐步发展成为肿瘤。

非急性转化反转录病毒也被用来寻找新的细胞原癌基因。研究人员大量收集由某一反转录病毒诱发的肿瘤组织,寻找其中共有的前病毒插入位点。这些共有的插入位点对于鉴定细胞原癌基因的激活具有重要提示作用。那些位于共有插入位点并且在肿瘤细胞中高度表达的细胞基因随之即被确认为原癌基因。如通过对小鼠乳腺肿瘤病毒(murine mammary tumor virus, MMTV) 引起乳腺癌的研究,发现了一些原癌基因,这些基因最初被称为 *int-1*, *int-2* 和 *int-3*, 现在则被统一命名为 *wnt* 基因[27]。Wnt 信号通路随后被发现在正常细胞发育和癌症细胞异常增殖过程中发挥了重要作用。前述 M-MuLV 插入激活的原癌基因 *pim-1* 也被发现是病毒在 T 细胞淋巴癌中的一个常见插入位点。最新的基因组学技术(高通量 DNA 测序技术) 在非急性转化反转录病毒诱导肿瘤研究中的应用加快了细胞原癌基因的发现速度[21]。基因组学技术能够迅速鉴定出肿瘤细胞中共有的前病毒插入位点,加速了新的原癌基因的发现。

不同种类的非急性反转录病毒能特异性地诱导不同类型肿瘤的发生,这种特异性主要由病毒的 LTR 决定。如在小鼠中 M-MuLV 能诱导 T 细胞淋巴癌的发生,而 F-MuLV 则导致红细胞白血病的发生。通过分子克隆技术将 2 种病毒的 LTR 区域进行交换,诱导产生的肿瘤类型会伴随着 LTR 的改变而发生变化。事实上,仅仅将位于 LTR U3 结构域中的 F-MuLV 增强子序列插入 M-MuLV,这种重组病毒的感染就能导致红细胞白血病的发生,反之亦然[22]。细胞和病毒的增强子通常都具有组织细胞特异性,只能与存在于特定类型细胞中的转录因子结合。因此反转录病毒的增强子可在某个特定分化类型的细胞中活化,并高效激活该细胞中的原癌基因,最终导致肿瘤的发生。

4 非病毒诱生肿瘤中原癌基因的激活

通过对反转录病毒研究,发现细胞原癌基因在肿瘤发生机制中的作用变得越发重要,尤其是发现在自然发生的人类肿瘤中(不存在反转录病毒感染) ,原癌基因也能被激活。有几种不同的机制会导致细胞原癌基因的激活。一种为在有些肿瘤中正常的原癌基因蛋白高表达,导致这一现象有 2 种可能: 其一,在某些肿瘤,染色体中含有原癌基因的区域会被大量扩增,这些区域通常为染色体的均质染色区(homogeneously staining region) 或微卫星区域(micro-satellite) 。如原癌基因 *her-2 /neu* 在某些亚型的乳腺癌和卵巢癌中会大量扩增[31]。事实上,高表达 *her-2 /neu* 的肿瘤临床预后更差。利用这一特性,可以把 *her-2 /neu* 的表达水平作为乳腺癌和卵巢癌肿瘤分级的指标之一。目前针对 *her-2 /neu* 的特异性靶向药物 Herceptin(一种单克隆抗体) 在治疗具有该基因扩增现象的肿瘤中特别有效[32]。其二,正常的原癌基因蛋白由于发生染色体易位(chromosomal translocation) 而高表达。某些类型的肿瘤有特征性易位,且往往涉及原癌基因的易位。Burkitt 淋巴瘤中存在特征性的 8 ;21 染色体易位,导致原癌基因 *c-myc* 转移至免疫球蛋白重链结构基因附近[9]。由于 Burkitt 淋巴瘤是 B 细胞的肿瘤,这一易位使得 *c-myc* 基因位于一个高度表达的基因附近,从而使 c-Myc 蛋白也发生高度表达。原癌基因在人类肿瘤中的另一激活机制涉及原癌基因蛋白自身的改变,如原癌基因 *ras* 在肿瘤中的激活由单核苷酸突变引起。Ras 蛋白是一个小 G 蛋白,在信号转导中发挥重要作用。Ras 与 GTP 结合后即被激活,结合的 GTP 被水解成 GDP 则又导致失活,如此循环往复。在肿瘤中由单个氨基酸置换而引起的 Ras 蛋白突变会导致 GTPase 活性的丧失[26]。突变的 Ras 蛋白不能将与之结合的 GTP 水解为 GDP,因此被持续地锁定在激活状态,导致持续性产生细胞

增殖信号。还有一种原癌基因活化机制是由于发生染色体易位而导致原癌基因发生改变。如 Philadelphia 染色体是慢性髓细胞性白血病(chronic myelogenous leukemia, CML)和某些特定 B 细胞白血病的特征性染色体易位。该易位导致新的融合转录产物的产生,一条染色体上的 *bcr* 序列与另一条染色体上的原癌基因 *c-abl* 发生融合[11,15],Bcr-Abl 融合蛋白的表达,使得 c-Abl 酪氨酸特异性蛋白激酶活性升高。最近研制开发的 Bcr-Abl 蛋白激酶小分子抑制剂 Imantinib 已成为治疗 CML 的一线药物。

5 Micro-RNA

RNA 干扰(RNA interference, RNAi)是新近发现的一种基因表达调控机制。最近还发现了一种经由较大的细胞转录产物加工而来的小双链微小 RNA(micro-RNA)[3]。micro-RNA 通过 RNAi 机制下调细胞 mRNA 的表达水平,以此调控其靶基因的活性。第 1 个 micro-RNA 是在研究禽类非急性转化反转录病毒诱导鸡 B 细胞淋巴癌产生的过程中首先被发现的[4]。对这些肿瘤的研究发现前病毒 DNA 整合于共有插入位点 *bic* 附近。病毒的插入导致 *bic* 转录产物过量表达,提示 *bic* 可能是一潜在的原癌基因。然而,研究表明 *bic* RNA 中不存在任何能编码蛋白的开放读码框架。当时无法解释该现象,成为困扰研究人员多时的神秘谜团[35]。在 RNAi 及 micro-RNA 被发现后,通过研究确认 *bic* 能编码一 micro-RNA,并将其命名为 mir155[20]。mir155 在正常的淋巴发育过程中起非常重要的作用,其高表达会导致淋巴癌的发生。

6 其他反转录病毒致癌机制

除上述反转录病毒的主要致癌机制外,有小部分反转录病毒会利用一些特殊机制诱发肿瘤形成。

6.1 人 T 细胞白血病病毒和牛淋巴肉瘤病毒

人 T 细胞白血病病毒(human T-cell leukemia virus, HTLV)和牛淋巴肉瘤病毒(bovine lymphosarcoma virus, BLV)属于 δ反转录病毒家族,分别引起成年人 T 细胞白血病(adult T-cell leukemia, ATL)和 B 细胞淋巴癌。HTLV 有 2 种主要的亚型:HTLV-Ⅰ和 HTLV-Ⅱ,其中只有 HTLV-Ⅰ与疾病相关。全世界约 2 000 万人感染 HTLV-Ⅰ,日本、加勒比地区、拉丁美洲和非洲是感染高发区。在这些地区,由于病毒感染率较高,ATL 也更为常见。HTLV-Ⅰ还引起神经系统疾病,如 HTLV 相关的脊髓疾病(HTLV-associated myelopathy, HAM),也被称为热带痉挛性瘫痪(tropical spastic paraparesis, TSP)等。

HTLV-Ⅰ诱发 ATL 的效率相对较低,且潜伏期更长(通常要 20 年以上),发病率每年为 1% ~2%。与典型的反转录病毒相比,HTLV 和 BLV 均携带额外的遗传元件 X 区域。这一区域可通过 mRNA 的可变剪切表达出数种病毒蛋白,包括 1 个反式转录激活元件 Tax 和 1 个胞质内未剪切病毒 RNA 的辅助蛋白 Rex。Tax 能反式激活 HTLV 的 LTR 区域以及一些细胞基因的启动子,包括白细胞介素 2(interleukin-2, IL-2)和 IL-2 受体(interleukin-2 receptor, IL-2R)。HTLV-Ⅰ的 Tax 蛋白能在体外转化培养的鼠成纤维细胞,具有癌基因的功能,而且该蛋白在 HTLV-Ⅰ诱导人原代 T 细胞永生化的过程中是必需的。T 细胞的永生化可能是 Tax 蛋白诱导产生的 IL-2 和 IL-2R 在同一细胞中共同表达所引起的[39],导致细胞产生由 IL-2 自分泌循环驱动的细胞分裂。因此 Tax 蛋白能使 HTLV-Ⅰ在感染的 T 细胞中建立前白血病状态并持续多年。然而奇怪的是,当 ATL 肿瘤形成后,肿瘤细胞即使仍含有 HTLV-Ⅰ,前病毒也不再表达 Tax 蛋白,似乎在肿瘤发展过程中存在针对 Tax 蛋白表达的选择机制。

另一方面,ATL 肿瘤细胞会表达另一 HTLV-Ⅰ蛋白 HBZ[14]。该蛋白是一 B-zip DNA 结合蛋白,由病毒负链编码,从下游 LTR 起始转录。HBZ 在所有 ATL 肿瘤中均有表达,提示其在 ATL 肿瘤发生过程中起重要作用。因此,2 个不同的 HTLV-Ⅰ蛋白参与了 ATL 的发生、发展过程。Tax 蛋白参与肿瘤的起始过程,而 HBZ 蛋白则参与肿瘤细胞转化的维持过程。

6.2 Jaagsiekte 绵羊反转录病毒

Jaagsiekte 绵羊反转录病毒(Jaagsiekte sheep retrovirus, JSRV)是 β反转录病毒家族的一员,能在绵羊中引起具传染性的绵羊肺腺瘤病(ovine pulmonary adenocarcinoma, OPA)。OPA 是肺泡分泌型上皮细胞、Ⅱ型肺细胞以及支气管 Clara 细胞形成的肿瘤。Ⅱ型肺细胞的正常生理功能是产生肺表面活性物质,以维持肺泡对于气体的渗透性。处于疾病终末期的动物会因肿瘤细胞分泌过量的表面活性物质而导致呼吸窘迫,在这些动物体内收集到的肺液(lung fluid)含有感染性病毒颗粒。据此认为病毒感染以及肿瘤可通过吸入肺液飞沫而传播。

当 JSRV 被分子克隆后, 对其基因组测序发现该病毒具有典型的反转录病毒基因(*gag*, *pol* 以及 *env*) , 并且不存在与细胞基因具有同源序列的病毒癌基因, 提示 JSRV 可能通过 LTR 激活原癌基因来诱导肿瘤产生。然而, 对 1 株仅含有单拷贝前病毒 DNA 序列的 JSRV 相关肿瘤细胞系的研究发现, 病毒 DNA 整合于结构基因肺表面活性物质 A (pulmonary surfactant A) , 该基因并非原癌基因[12], 因此 LTR 激活原癌基因很可能并非是 JSRV 的致癌机制。JSRV 是一有效的致癌物, 将病毒经气管接种至新生羊即会导致 OPA, 平均潜伏期 6 周左右。有些动物甚至短至 2 周即能发病, 提示 JSRV 很可能自身即携带癌基因, 但对病毒 DNA 序列的分析未发现支持性证据。事实上当 JSRV 的 DNA 被转染入鼠成纤维细胞(如 NIH-3T3 或 208F 细胞) [25] 或上皮细胞(如大鼠 RK3E 细胞) 后[24], 会形成细胞转化灶, 转化的细胞具有致瘤性。最不同寻常之处在于 JSRV 的 *env* 基因足以引起细胞转化。因此, JSRV 的 Env 蛋白本身具有病毒癌基因的功能, 单独将 JSRV 蛋白通过腺相关病毒载体介导的方法导入 SCID 小鼠的肺部即能引起肺癌。

反转录病毒的包膜蛋白起初是以聚合蛋白前体的形式而被翻译的, 其中包含 2 个不同的包膜蛋白, 位于 N 端的表面蛋白(surface, SU) 和位于 C 端的跨膜蛋白(transmembrane, TM) 。SU 蛋白含有病毒受体的结合域, 位于病毒颗粒的外层, 而 TM 蛋白则跨越病毒颗粒的脂质双层, 并将 SU 锚定在病毒颗粒上。据推测在 JSRV 转化细胞或肿瘤细胞上病毒包膜蛋白存在相同结构, SU 蛋白位于细胞的表面而 TM 蛋白跨越质膜并将其 C 端暴露于胞质内。对 Env 蛋白的结构功能进行分析, 提示 TM 蛋白仅为 45 个氨基酸残基长度的胞质末端对于细胞转化是必需的[28]。推测其与细胞蛋白发生相互作用, 导致产生更多的细胞增殖信号。有 2 个信号通路对于 JSRV Env 蛋白的细胞转化非常重要: PI3K-AKT-mTOR 通路和 Ras-MEK-ERK 通路[24]。对于涉及 JSRV 转化过程的细胞蛋白寻找及鉴定, 目前仍在进行中。

JSRV Env 蛋白的其他部分对于致瘤性可能也具有重要作用。SU 蛋白能与细胞受体 hyaluronidase-2(Hyal-2) 结合(人和羊的 Hyal-2 是 JSRV 的受体, 而大鼠的 Hyal-2 不是) [29]。Hyal-2 在人类染色体上被定位于 3p21.3 区域, 这部分染色体也是肺癌中常见的缺失突变区域。肿瘤中染色体的缺失常伴随抑癌基因的丢失, 因此 JSRV 有可能通过干扰 Hyal-2 的肿瘤抑制功能而导致肿瘤发生。已有实验证据支持这一理论。在 1 株人肺上皮细胞系中, 发现 Hyal-2 蛋白与跨膜受体型的酪氨酸激酶 Stk(也被称为 RON) 组成一复合体[10]。当 Stk/RON 与 Hyal-2 形成复合体后, 其激酶活性被抑制。在细胞中表达 JSRV Env 蛋白, 导致 Hyal-2 与 JSRV SU 结合, 使 Stk/RON 从其抑制复合体中释放出来。因此, 在羊中 JSRV 诱发肿瘤的机制可能是通过 SU 蛋白与 Hyal-2 结合, 并增强 Stk/RON 信号而引起的; 然而在大鼠中, 因 JSRV 的 Env 并不与 Hyal-2 结合, 故还存在其他作用机制。

6.3 MMTV

MMTV 属于 β反转录病毒, 能诱发乳腺癌和 B 细胞淋巴癌。插入激活细胞原癌基因是 MMTV 诱导肿瘤产生的机制。然而, 最近发现 Env 蛋白对病毒致癌性也有作用。小鼠乳腺上皮细胞系被 MMTV 感染后, 当其在塑料平面上生长时并不表现出任何变化, 但当细胞在三维立体培养空间中生长, 悬浮于含有细胞外基质组分(matrigel) 的培养基时, 未被感染的细胞会形成极性化的上皮细胞微球[19], 感染细胞在 matrigel 中的生长具有异常表现, 会形成更大的细胞团且缺乏极性。MMTV 的 Env 蛋白是细胞发生这些变化的主要原因。Env 蛋白上的 ITAM 结构域对于细胞的生长变化具有重要的作用。

7 病毒与人类肿瘤紧密相关

在前言中提到, 大约 20% 的人类肿瘤是由病毒感染引起的。表 2 列出了目前已知的与肿瘤相关的各类病毒。在某些情况下, 这些病毒直接扮演了致癌因子的角色, 即病毒或其基因产物在细胞中的表达能直接导致肿瘤发生; 而在另外一些情况下, 病毒通过间接途径引起肿瘤(病毒并不存在于肿瘤细胞中) 。本文主要阐述反转录病毒诱发肿瘤的机制, 其他非反转录病毒在本文中并未涉及。

7.1 HTLV-Ⅰ

HTLV-Ⅰ及其在诱发 ATL 过程中的作用已在 6.1 部分中详细阐述。

7.2 人类免疫缺陷病毒 1 型

人类免疫缺陷病毒 1 型 (human immunodeficiency virus type 1, HIV-1) 是获得性免疫缺陷综合征 (acquired immunodeficiency syndrome, AIDS) 的病原体。由于病毒导致 AIDS 患者体内免疫系统发生缺陷, 肿瘤随之发生。因此, HIV-1 被认

为能间接诱导肿瘤产生。AIDS 相关的肿瘤包括 Kaposi 肉瘤、AIDS 淋巴癌及宫颈癌。有趣的是，AIDS 相关肿瘤都是由于感染了其他致癌病毒引起的。HIV-1 感染引起的免疫缺陷导致 AIDS 患者对致癌病毒感染的抵抗力下降，从而间接引起肿瘤。事实上与病毒感染无关的肿瘤（如肺癌）的发生率在 AIDS 患者中并没有显著变化。

7.3　异嗜性小鼠白血病病毒相关病毒

最近发现了一种新的人类反转录病毒——异嗜性小鼠白血病病毒相关病毒（xenotropic MuLV-related virus, XMRV），该病毒是在家族性前列腺癌患者体内首先发现的，这些患者的先天免疫功能具有缺陷[36]。RNase L 是干扰素抗病毒机制中的一个效应蛋白。具有 R462Q 纯合突变的个体发生前列腺癌的风险会提高 2 倍，推测这些个体对导致前列腺癌的病毒感染的易感性更高。约 40% 的肿瘤细胞显示被一新型的反转录病毒 XMRV 感染。XMRV 与 MuLV 非常相似，其感染可能是一类跨物种现象，人被小鼠反转录病毒感染。对于 XMRV 的研究仍处于起步阶段，该病毒在人类前列腺癌中的作用及致癌机制还不清楚。

7.4　内源性反转录病毒

反转录病毒生活史中的一个重要特征在于病毒 DNA 会被整合入宿主染色体。如果反转录病毒感染发生在即将形成胚胎的生殖细胞中，那么被感染的子代会将反转录病毒的前病毒作为一标准的遗传元件传递到后代。这种可遗传的前病毒被称为内源性反转录病毒（endogenous retrovirus, ERV）。在进化过程中这一现象频繁发生，大约 8% 的人类基因组由内源性反转录病毒组成。绝大多数的 ERV 是复制缺陷型的，极有可能反映了机体对于具有复制能力的 ERV 选择压力。很多 ERV 能够编码功能蛋白，可能参与肿瘤的发生过程。在某些人类肿瘤中已发现 ERV 的表达，如精原细胞瘤[17]。近来，在小鼠中的实验证据也支持这一理论的假说，如 HERV-K 所编码小的病毒调节蛋白[23]能够促使鼠成纤维细胞转化成瘤[1]。

（田晓晨 译）