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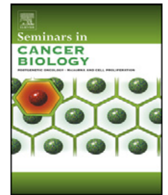

Review

T antigen transgenic mouse models

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ABSTRACT

The study of polyomavirus has benefited immensely from two scientific methodologies, cell culture and *in vitro* studies on one side and the use of transgenic mice as experimental models on the other. Both approaches allowed us to identify cellular products targeted by the viruses, the consequences of these interactions at the phenotypic and molecular level, and thus the potential roles of the targets within their normal cellular context. In particular, cell culture and *in vitro* reports suggest a model explaining partially how SV40 large T antigen contributes to oncogenic transformation. In most cases, T antigen induces cell cycle entry by inactivation of the Rb proteins (pRb, p130, and p107), thus activating E2F-dependent transcription and subsequent S-phase entry. Simultaneously, T antigen blocks p53 activity and therefore prevents the ensuing cell-cycle arrest and apoptosis. For the most part, studies of T antigen expression in transgenic mice support this model, but the use of T antigen mutants and their expression in different tissue and cell type settings have expanded our knowledge of the model system and raised important questions regarding tumorigenic mechanisms functioning *in vivo*.

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1. Introduction

Two major very exciting areas of scientific research came together in the early 1980s and resulted in the production of models to investigate cancer. On one hand, the technology to produce transgenic mice was being developed rapidly [1] and, at the same time, many viral and cellular oncogenes were being discovered and subsequently cloned. It soon became evident that the expression of SV40 sequences including the large T antigen (T antigen) in mice resulted in tumor development in particular tissues [2,3]. Up until that point cancer models had been based on cell lines obtained from human or animal tumors and maintained in tissue culture, or from the subcutaneous inoculation of those cells into immunodeficient mice. Although obviously useful, these models did not offer a good approach to understanding the genetics and the molecular pathways involved in different types of tumorigenesis, nor to the relevance to particular tumors and their site of origin "*in vivo*". The arrival of transgenic mice expressing T antigen made clear, not only that T antigen expression was capable of driving resting cells into active proliferation status, but also that this trait was hereditary and the mouse progeny presented cancer predisposition. As a result, SV40 T antigen has been expressed in multiple tissues of transgenic mice to mimic tumorigenic processes.

T antigen has been expressed under the control of numerous promoters directing its expression to different cells and/or tissue types, including mammary glands, pancreas, liver, prostate, salivary glands, intestine, brain, lung, kidney, eye, smooth muscle, cartilage and bone [4,5]. Some particular systems are of special interest due to their clinical relevance. For instance, the phenotype induced by expression of T antigen in the prostate closely resembles specific tumorigenic conditions in humans. In other instances, the exhaustive characterization of transgenic mice expressing full length T antigen and mutant derivatives of the oncogene have helped to dissect the molecular mechanisms that must be disrupted in a normal cell in order for it to progress through different stages of tumorigenesis (e.g. pancreas, intestine, choroid plexus epithelium, eye lenses). Space constrains force us to concentrate on these last systems.

2. The model system

SV40 depends heavily on the cell machinery to pursue its own genome replication and transcription, as the viral genome is relatively small. In particular, the virus requires that the host cell re-enters S-phase in order to use the cellular machinery for its own replication. Although not a normal part of the viral life cycle, the viral DNA can sometimes integrate into the chromosome of a non-permissive species and maintain the expression of the viral T antigens, and transformation of the host cell follows. Upon transformation with SV40, cells become capable of proliferation and survival in a number of environments that are otherwise growth-restrictive for non-transformed cells. For instance, transformed cells can grow in the absence of serum, form dense foci of multilay-

Abbreviations: T antigen, SV40 large T antigen; CPE, choroid plexus epithelium.

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ered cells in culture, grow independently of anchorage, or induce tumors when injected into immuno-compromised mice.

Although the full transformation potential of SV40 is mediated by two virus-encoded proteins – the 708 amino acid large T antigen and the 174 amino acid small t antigen – large T antigen "per se" is necessary and often sufficient to induce and maintain transformation. The virus needs this extremely versatile protein for DNA replication, transcriptional control and virion assembly. These functions are carried out through the independent and cooperative action of five structural domains in T antigen, which include a J domain at the amino-terminus, a sequence-specific DNA binding domain (OBD, for origin binding domain), a Zn-binding domain, an ATPase domain (AAA+), and the host-range domain (Fig. 1).

In addition, T antigen is able to induce transformation in cell culture and *in vivo* transgenic systems, a phenomenon effected through its interaction with at least five cellular targets: hsc70, the three Rb tumor suppressor proteins (pRb, p107, and p130), and the tumor suppressor p53 [4,6]. T antigen binds p53 through interactions with exposed amino acids on the surface of its ATPase domain [7]. Similarly, the three Rb-proteins bind to an LXCXE motif located in the flexible linker between the J domain and the OBD. Finally, the J domain governs recruitment and activation of hsc70, a cellular chaperone [8–10]. T antigen has been shown to interact with another three targets, and these interactions could contribute to transformation as well, but they have been less studied. Two of these factors, the checkpoint kinase Bub1 and the cullin Cul7 interact with T antigen via the flexible linker near the LXCXE motif [11–14] (Fig. 1). Finally, the transcriptional adapter proteins CBP/p300 [15–17], bind T antigen through interactions with p53 and could also contribute to transformation. In addition, small t antigen contributes to transformation through its interaction with the cellular phosphatase PP2A [18,19].

The study of T antigen in cell culture systems has clearly established that the ability of this oncogene to re-activate the cell cycle and to transform multiple cell types is linked to its capacity of tampering with the Rb family, as well as its ability to prevent the ensuing apoptosis by binding and inactivating the tumor suppressor p53. Both functions then allow uncontrolled cell proliferation. Nevertheless, some exceptions are found in tumorigenesis induced by T antigen in transgenic systems, which will be discussed below.

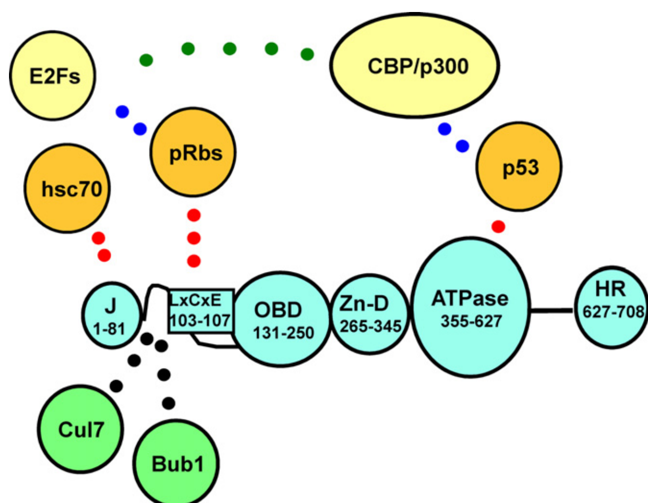

Fig. 1. Domain structure of SV40 T antigen. Different protein domains of T antigen (blue) and the corresponding amino acids are indicated: J domain (J); Rb-protein binding region (LXCXE); origin binding site (OBD); Zinc finger domain (Zn-D); ATPase region contaning the p53 binding region (ATPase); host-range domain (HR). Established cellular targets are depicted in orange and their regions of interaction with T antigen indicated (red dots). Known interacting proteins whose role has not been established by transgenic analysis are depicted in green. Cellular components (yellow) that might associate with T antigen through other proteins as well as their possible interactions are also indicated.

3. Tissue system

3.1. Brain

SV40 T antigen has been used to induce tumorigenesis in several cell types of the brain although, by far, the most studied is the choroid plexus epithelium (CPE). Located within the brain ventricles, the choroid plexus is a continuous epithelial layer that produces and filters the cerebrospinal fluid, in fact cushioning and protecting the brain from foreign substances, immune cells and/or metabolic waste. The continuous epithelial layer lining the ventricles contain multiple tight junctions on the apical surface of the cells, thus creating an effective blood-cerebrospinal fluid barrier.

Regarding T antigen function, the CPE is a particularly interesting system, as the expression of different T antigen mutants in this tissue have yielded numerous insights into the molecular mechanisms necessary to disrupt quiescence and re-enter the cell cycle. Expression of T antigen in the CPE induces the formation of rapidly developing tumors, a phenotype that does not require the expression of small t and results in the death of the mice prior to or around 1 month of age [20,21]. If an amino-terminal truncated version of T antigen (dl137, T121) is expressed in the CPE, similar tumors arise but at a much lower rate [20,22]. A mutant T121 protein carrying a defective LXCXE motif, and thus unable to interfere with the Rb pathway, is unable to induce tumors, indicating that blocking the Rb pathway is essential to induce CPE proliferation [22]. The fact that tumors arise at a much slower pace in T121-expressing animals indicates that the progression of CPE tumorigenesis is linked to a cellular function targeted by the carboxy-terminus end of T antigen [22]. Indeed, blocking the p53-mediated apoptosis induced by T121 allows for fast tumor proliferation [23], and upregulation of the transcriptional activator E2F1 in response to pRb inactivation seems to be required to induce p53 and the subsequent apoptosis [24].

Therefore, at least in the CPE, disruption of the Rb pathway by T antigen releases the brakes that halt cell proliferation in quiescent cells. Abnormal entry into S-phase and upregulation of E2F1 then trigger a p53-mediated response aimed to eliminate the abnormally proliferating cells, but T antigen is also capable of preventing this apoptosis by binding and inactivating p53 itself, thus inducing fast and aggressive tumors.

Atm (ataxia telangiectasia mutated) is a serine/threonine protein kinase that phosphorylates several proteins in response to DNA damage. This phosphorylation initiates a series of events leading to cell cycle arrest, DNA repair or apoptosis. In particular, the Atm function upstream of p53 has been shown to be essential to control the apoptosis induced by DNA damage. Nevertheless, the removal of Atm does not seem to be affect the apoptosis induced by T antigen in the CPE [25]. Thus, the apoptotic responses triggered by different stimuli (e.g. DNA damage versus oncogenic stimuli), despite being mediated by p53, do not necessarily utilize the same molecular mechanisms.

Other cell types in the brain have been used to investigate oncogenesis mediated by T antigen. For instance, conditional expression of the truncated T mutant T121 in astrocytes leads to aberrant proliferation and extensive apoptosis [26]. Surprisingly and in contrast with the phenotype induced in CPE cells, development of astrocytomas is not dependent on p53, but instead is affected by the amount of the tumor suppressor PTEN present [26]. Thus, in this case, the apoptosis mediated by T antigen does not rely on p53, and T antigen triggers other signals in the cell destined to counteract its abnormal effects on cell proliferation.

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