Seminars in Cancer Biology 19 (2009) 218–228

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Cellular transformation by Simian Virus 40 and Murine Polyoma Virus T antigens

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ARTICLE INFO

Keywords:
SV40
Polyoma
T antigen
p53
Retinoblastoma
Src
PI3 kinase
Shc

ABSTRACT

Simian Virus 40 (SV40) and Mouse Polyoma Virus (PY) are small DNA tumor viruses that have been used extensively to study cellular transformation. The SV40 early region encodes three tumor antigens, large T (LT), small T (ST) and 17KT that contribute to cellular transformation. While PY also encodes LT and ST, the unique middle T (MT) generates most of the transforming activity. SV40 LT mediated transformation requires binding to the tumor suppressor proteins Rb and p53 in the nucleus and ST binding to the protein phosphatase PP2A in the cytoplasm. SV40 LT also binds to several additional cellular proteins including p300, CBP, Cul7, IRS1, Bub1, Nbs1 and Fbxw7 that contribute to viral transformation. PY MT transformation is dependent on binding to PP2A and the Src family protein tyrosine kinases (PTK) and assembly of a signaling complex on cell membranes that leads to transformation in a manner similar to Her2/neu. Phosphorylation of MT tyrosine residues activates key signaling molecules including Shc/Grb2, PI3K and PLCγ1. The unique contributions of SV40 LT and ST and PY MT to cellular transformation have provided significant insights into our understanding of tumor suppressors, oncogenes and the process of oncogenesis.

1. Introduction

Murine polyomavirus (PY), the founding member of the *Polyomaviridae* family was identified in the 1950s. As the name implies, PY causes a wide variety of tumors. SV40 was discovered in 1960 as a vacuolating virus contaminating rhesus monkey kidney cell cultures used to produce the poliovirus vaccine [1]. These discoveries were quickly followed by reports that SV40 could form tumors in newborn rodents and transform primary human cells. These initial reports led to nearly 50 years of intense investigation into the biology and oncogenic potential of these viruses.

These viruses have been important models for study of DNA and RNA metabolism. Our appreciation of the compact and efficient SV40 promoter and origin of replication as well as mRNA splicing and polyadenylation signals led to their widespread use in many mammalian expression vectors. However, the greatest gains have come from studies on T antigen-mediated neoplastic transformation and tumorigenesis that apparently reflect side effects of viral signaling needed for infection and replication. Study of SV40 led to discovery of the tumor suppressor p53 and insight into the structure and function of the retinoblastoma tumor suppressor Rb. Similarly, the study of PY led to discovery of phosphotyrosine kinases (PTK) and phosphoinositide 3-kinase (PI3K) signaling.

Proteins encoded by the early region of the two viruses are responsible for transformation. Splicing of primary early transcripts takes place in both viruses, but in ways that generates different kinds of gene products. Importantly, each of the T antigens functions in different cellular compartments reflecting their unique contributions to the virus life cycle and to cellular transformation.

The SV40 viral early region encodes three proteins LT, ST and 17KT expressed from the early promoter after differential splicing (Fig. 1). The three T antigens share the N-terminal 82-residues that contains a DnaJ or J domain (Fig. 1). ST continues in the same open reading frame to encode a 174-residue protein. LT and 17KT use the same intron that skips the ST unique region and share residues 1–131. LT continues to residue 708. 17KT has a second intron followed by a third exon that encodes just 4 additional residues to encode a 135-residue protein.

The primary transcripts of the PY early region are also differentially spliced to produce LT, MT and ST with a common N-terminal, 79-residue, J domain (Fig. 1). ST shares an additional 112-residues

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doi:10.1016/j.semcancer.2009.03.002

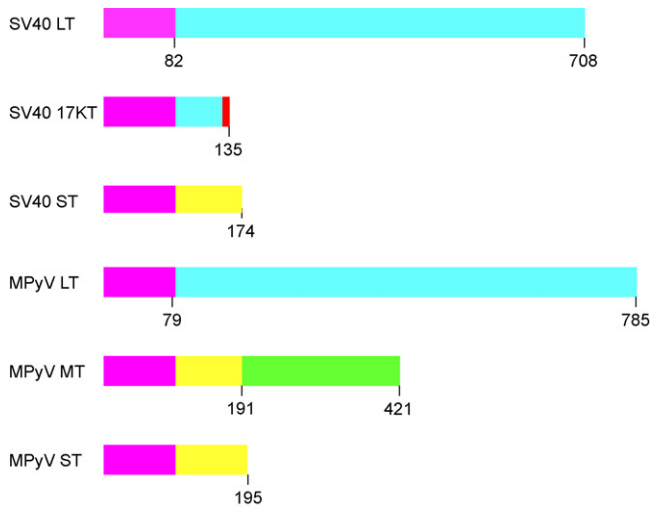

Fig. 1. SV40 and Mouse Polyoma T antigens. Large (LT), 17KT and Small (ST) of SV40 and PY as well as Middle (MT) T antigens from PY share coding regions. The N terminal 82 (SV40) and 79 (PY) residues for all T antigens are identical (magenta). SV40 LT and 17KT share residues 83–131 (blue). The last 4 residues of SV4017KT are unique (red). PY MT and ST share residues 80–191 (yellow). PY MT has a unique domain comprising residues 192–421 (green).

with MT. The C-terminal region of each protein is unique and determines their subcellular location. Similar to SV40, PY LT is nuclear and ST is predominantly cytoplasmic. PY MT is anchored to membranes.

LTs from both viruses have essential roles in viral DNA replication. SV40 LT also provides most of the transforming activity. For example, while both SV40 and PY LT have a helicase domain, this SV40 LT domain also serves to bind to p53. PY MT provides nearly all of the transforming functions for PY and also plays a role in viral replication. PY MT activates PTK signaling in a manner similar to that induced by the cellular receptor tyrosine kinase Her2/neu. ST serves similar but not identical roles in these two viruses and cooperates with the other T antigens to transform cells.

It is not clear why SV40 and PY have such distinct transforming mechanisms. A similar species-specific dichotomy is seen in papillomavirus, where the bovine BPV E5 activates tyrosine kinase signaling and human HPV E6 inactivates p53 and not conversely. A reasonable hypothesis is that the viruses take advantage of unique species or tissues differences, such as transcription factors, for viral growth.

2. Dissociation of SV40 LT replication from transformation functions

Since SV40 LT provides essential functions for viral DNA replication that can ultimately cause lysis of the infected cell, these replication functions must be disabled for LT to promote long-term cellular transformation and tumorigenesis. Infection of permissive monkey cells typically results in rapid viral replication followed by lysis or vacuolation of the host cell and subsequent release of progeny. SV40 can infect non-permissive rodent cells and semi-permissive human cells, but the transformed state lasts for a few days before reverting to a normal phenotype in a process known as abortive transformation although a stable transformed cell may appear on rare occasion. Notably, primary human mesothelial cells that can support low levels of SV40 replication with persistence of episomal SV40 DNA and a transformed phenotype [2]. SV40 transformation can be readily studied in rodent and human cells by transfection of viral DNA or with retroviral vectors encoding LT and ST [3].

The ability of SV40 to transform cells reflects a summary of several distinct contributions. LT can immortalize primary cells, reduce serum requirements for growth, enable cells to overgrow a monolayer of cells and form foci, support anchorage independent growth and induce tumor formation when cells are implanted as xenografts in nude mice. There is a hierarchy of these transformed features with immortalization and growth in reduced serum as the least stringent and anchorage independent growth and tumor formation as more stringent. In general, immortality or unlimited growth in vitro is a prerequisite for malignant transformation or unlimited in vivo growth. There is often a high degree of correlation between anchorage independent growth and growth as xenografts [3].

The temperature sensitive SV40 LT mutant tsA58, containing the point substitution A438V, has been useful for defining the contribution of LT to transformation and immortalization. Stable expression of tsA58 LT at the permissive temperature (32–33.5 °C) is necessary for continued proliferation of rodent and human fibroblasts [4]. Shifting cells to the restrictive temperature (>39 °C) reduces the stability of the tsA58 LT and eliminates its ability to bind to p53 resulting in growth arrest, senescence and crisis [5]. The tsA58 LT has also been used to generate several different transgenic mouse and rat strains enabling normal development at the restrictive temperature in vivo while permitting immortalization of various tissues when cultured at the permissive temperature in vitro [6].

3. Domains of SV40 LT

LT forms a hexameric structure with the oligomerization domain (residues 251–627) containing ATPase and helicase activities that participate in viral DNA replication [7]. The outside surface of each subunit of the LT hexamer serves to bind to p53 that in turn recruits p300 and CBP [8]. LT interaction with host replication proteins including DNA polymerase α and replication protein A is required for LT-mediated viral replication but not for LT-mediated transformation.

4. J domain

The N-terminal domain of SV40 LT and ST forms a J domain, a highly conserved structural domain present in the DnaJ/Hsp40 family of molecular chaperones. The J domain of SV40 LT binds specifically to Hsc70 and activates its ATPase activity (Fig. 2) [9,10]. The HPDK residues in the J domain of LT are required for this activity. Conversely, a variety of non-functional LT mutants can bind to

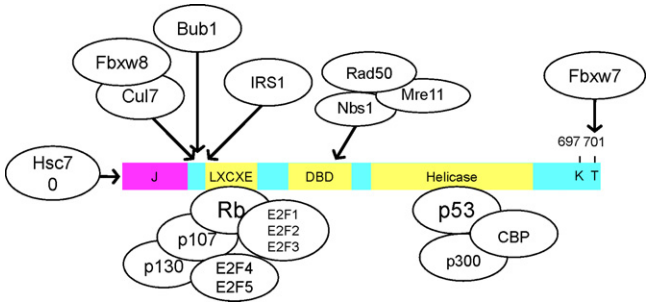

Fig. 2. SV40 LT binding proteins. SV40 LT binding proteins and their approximate binding regions in LT. Hsc70 binds specifically to the N-terminal DNA J domain. The Cullin ring ligase complex containing Cul7, Fbxw8 as well as Rbx1 and Skp1 (not shown) bind to residues between 69 and 102. Bub1 requires residues 89–98 and IRS1 binds to an N-terminal domain. The Rb-related proteins including p107 and p130 bind to the LXCXE motif encoded by residues 103–107. The E2F proteins and DP1 heterodimeric partner bind to the Rb proteins. The MRN complex containing Mre11, Rad50 and Nbs1 binds to the DNA binding domain (DBD). The p53 tumor suppressor binds to the Helicase domain and requires residue 350 through 627. The F-box containing protein Fbxw7 binds to the phosphorylated residue T701.