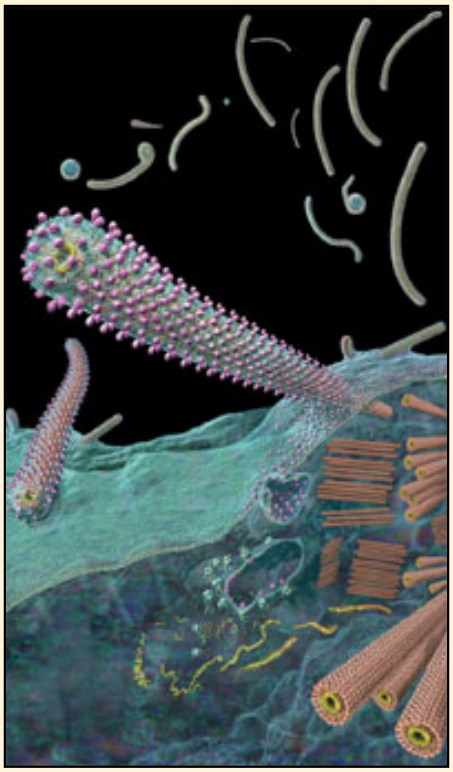
The Scientist
www.the-scientist.com

TSG101: An Antiviral Target with a Murky Past

Viruses hijack a host protein that normally sorts the trash |

By Ricki Lewis



USURPING THE CELLULAR WHEELMAN: Ebola (above) is a killer virus that may commandeer cellular protein-sorting pathways through TSG101.

Viruses can't do much without a host. They propagate by usurping cellular machinery, and much of an infection's misery is due to the immune response. But conventional therapies, without much luck, essentially have targeted one-half of the infection equation: the foreign invader. Now, researchers increasingly are setting their sights on the host's role.

Investigators recently attempted to mitigate the host immune response and the associated collateral damage (see sidebar below). Some explore a complementary approach: to cut off access to the cellular pathway that a virus commandeers to mass-produce, specifically, the route used to bud from the cell. Doing so, without impairing the host, could provide a powerful new broad-spectrum antiviral agent. "Conventional antivirals are highly specific, and pathogens can mutate to insensitivity," says Stanley

Cohen, professor of genetics and medicine at Stanford University. "But if one can attack host genes required for pathogens to be pathogenic, resistance is less likely. One can affect the pathogenicity of the virus."

DUBIOUS BEGINNINGS Cohen, who with Herbert W. Boyer in the early 1970s essentially founded recombinant DNA technology, says he might have zeroed in on what may become the first such "host-oriented" therapeutic target: Tumor Susceptibility Gene 101. Despite its moniker, TSG101 may have less to do with cancer than first thought, and everything to do with the vacuolar protein-sorting pathway that ferries excess cell-surface receptors to lysosomes, the enzyme-filled sacs that dismantle debris. Certain killer viruses, including HIV, Ebola, and Marburg, hijack this transport system using TSG101 protein to cloak themselves in membranous escape pods. At least three biotech companies are developing drugs to target TSG101, which Cohen dubs "the getaway driver for viral release." But a clinical future awaits clarification of its obfuscated origins as a tumor suppressor.

Discovering TSG101 in 1996 was the first fruit of random homozygous knockout (RHKO) technology, an antisense technique that identifies genes by a particular function, in this case, cellular transformation.[1] Dampening TSG101 expression transformed murine 3T3 fibroblasts, which caused metastatic tumors in nude mice. The encoded protein bore the hallmarks of a transcription factor, but the links to carcinogenesis turned out to be indirect.

THE VIRAL PIRATE In 2000, experimental roads converged to flesh out the role of TSG101. Beth Agresta, a graduate student working with molecular geneticist Carol Carter, State University of New York, Stony Brook, was screening for cellular proteins that interact with HIV proteins. "She got several hits and wrote her thesis on one, but continued to check the database to see if there was any more information. One day, there was," recalls Carter. That was TSG101. Carter and Cohen collaborated to get at protein function.

Soon, another graduate student in Carter's group, Lynn VerPlank, identified the major viral core protein Gag as HIV's binding site for TSG101.[2] Gag lets mature viruses bud from cells. At about the same time, Jennifer Garrus, a graduate student with biochemist Wesley Sundquist, University of Utah School of Medicine, Salt Lake City,

blocked TSG101 with small interfering RNA, halting HIV budding. When she restored gene function, budding resumed.[3]

Gag and TSG101 attach by ubiquitin tags, and the host protein then escorts the viral emissary into the vacuolar protein-sorting pathway. But instead of being carted away to digestive doom in the lysosome, Gag proteins attach by their bound TSG101s to bits of cell membrane, ultimately becoming packaged into mature virions.

Yeast provided another connection. Also in 2000, University of Utah biologist Markus Babst and Scott Emr, a Howard Hughes Medical Institute investigator at the University of California, San Diego, found that TSG101 has a yeast counterpart, Vps23.[4] Vps23/TSG101 is a subunit of a complex, called ESCRT-I, which binds and sorts ubiquitin-tagged molecules. They tested Cohen's TSG101 mutant cell line for protein transport defects and found trafficking phenotypes, says Babst.

The solid link to intracellular protein transport may explain the dual role of TSG101 in carcinogenesis and viral infection. The pathway normally recycles cell-surface receptors, including growth-factor receptors. Silence TSG101, and these receptors aren't sent to the lysosomal garbage dumps; instead they accumulate at the cell surface like uncollected trash. There, the receptors transduce too many signals to divide, and runaway mitosis results.

But the cancer connection still is not as clear as some researchers would like. "The role of TSG101 in cancer has been quite elusive," says Sundquist, citing a retracted report on TSG101 mutations in cancer patients.[5] And Kay-Uwe Wagner, at the Eppley Institute for Research in Cancer and Allied Diseases at the University of Nebraska Medical Center, Omaha, used knockout mice to show that TSG101 is essential for embryo implantation but not tumor suppression.[6] She also reexamined the original 1996 tumorigenic cell line and found "whopping levels" of TSG101.[7] She blames Cohen's use of an immortal cell line. "Nobody was able to repeat these transformation studies in other cell lines," she says.

DRUG DEVELOPMENT Still, Cohen maintains that TSG101 makes a good antiviral target. He and former postdoctoral researcher Limin Li cofounded the company Functional Genetics, which is developing a viral

budding inhibitor based on TSG101's action.

But the jump from promising target in cell culture to antiviral development will not be easy. Such a drug probably would block normal ESCRT function as well, says Babst. "This could result in a tumorigenic phenotype." Effects could be wide-ranging. "I'm worried about the potential that a TSG101 inhibitor would exhibit general cytotoxicity," suggests Sundquist, who also works with Myriad Pharmaceuticals in Salt Lake City.

Even if TSG101 never overcomes its tainted beginnings, it may still pioneer host-oriented therapeutics, for there may be unknown aspects of the viral subversion of TSG101 to exploit. Concludes Carter: "That's what one goes after--to understand the process, what the virus does to divert the machinery--and target that."

Ricki Lewis (rickilewis@nasw.org) is a freelance writer in Scotia, NY.

References

1. L. Li, S.N. Cohen, "Tsg101: A novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic loci in mammalian cells," *Cell*, 85:319-29, 1996.

2. L. VerPlank et al., "Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag)," *Proc Natl Acad Sci*, 98:7724-9, 2001.

3. J.E. Garrus et al., "Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding," *Cell*, 107:55-65, 2001.

4. M. Babst et al., "Mammalian tumor susceptibility gene 101 (TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking," *Traffic*, 1:248-58, 2000.

5. L. Li et al., "The TSG101 tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer," *Cell*, 93:661, 1998.

6. K. Wagner et al., "Tsg101 is essential for cell growth, proliferation, and cell survival of embryonic and adult tissues," *Mol Cell Biol*, 23:150-62, 2003.

7. A. Krempler et al., "Targeted deletion of the Tsg101 gene results in cell cycle arrest at G1/S and p53 independent cell death," *J Biol Chem*, 277:43216-23, 2002.