Comment on Masood et al, page 1310

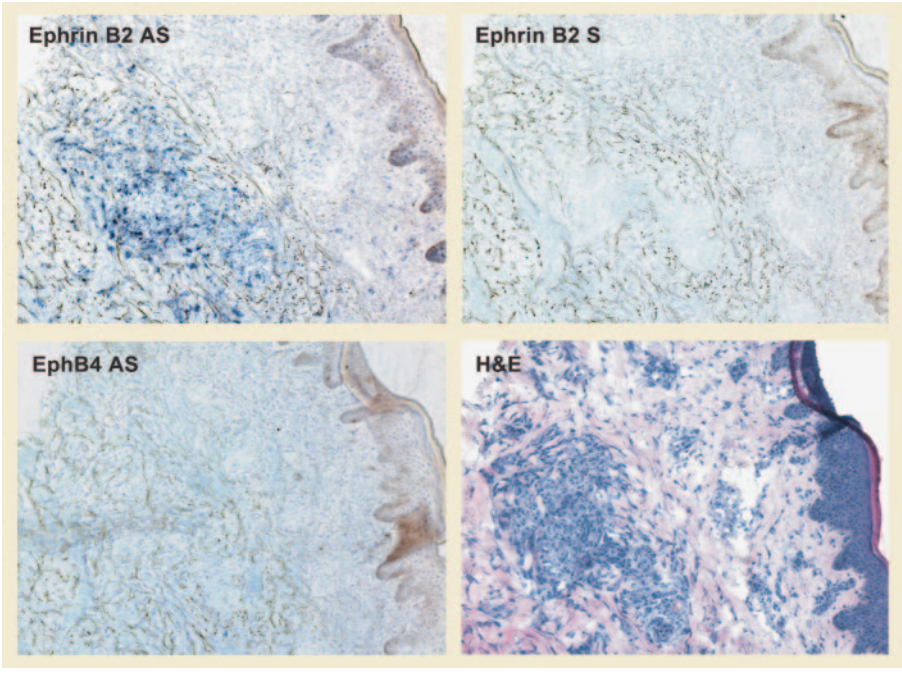
Talk amongst yourselves

William Wachsman VETERANS AFFAIRS SAN DIEGO HEALTHCARE SYSTEM AND UNIVERSITY OF CALIFORNIA, SAN DIEGO SCHOOL OF MEDICINE

The role of the HHV-8 vGPCR gene in KS pathogenesis is further explained by the finding that it induces ephrin B2 expression. That KS cell survival is dependent upon ephrin B2 raises broader questions about the biology and therapy of this tumor.

Human herpesvirus, type 8 (HHV-8) is the etiologic agent for all forms of Kaposi sarcoma (KS). The preponderance of evidence indicates that the viral G-protein–coupled receptor (vGPCR), a homologue of the CXC α-chemokine receptor family and related to the interleukin 8 (IL-8) receptor, is key to KS development. Experiments have demonstrated that vGPCR causes endothelial cell transformation and dermal lesions that have striking similarity to natural KS.[1] vGPCR is constitutively active, inducing expression of phosphatidylinositol 3–kinase (PI3K)/Akt and a variety of transcription factors thought to be integral to cell transformation and to perpetuation of the neoplastic state via autocrine/paracrine signaling.[2,3]

In the current issue, Masood and colleagues show that ephrin B2, but not EphB4, is expressed in KS spindle cells infected with HHV-8, thereby establishing an arterial phenotype. This phenotypic switch—uninfected endothelial cells express EphB4—is remarkable in terms of both biology and viral pathogenesis. They also demonstrate that vGPCR induces expression of ephrin B2 in endothelial cells, and they show that this action is mediated by several factors, including vascular endothelial growth factor (VEGF), IL-8, IL-6, and VEGF-C. These results suggest that ephrin B2 is the most distal element on a multipronged pathway whose activity is driven by vGPCR. Finally, this investigation reveals that KS cell viability in vitro is markedly diminished when ephrin B2 expression is decreased, likely at the hands of death receptor–mediated apoptosis. Thus, ephrin B2 is critical to establishing the phenotype and neoplastic properties of KS.



Ephrin B2 but not EphB4 is expressed in KS biopsy tissue. See the complete figure in the article beginning on page 1310.

These observations suggest that therapeutic targeting of vGPCR or factors downstream on the network through to and including ephrin B2 could result in tumor regression. But as in *Saturday Night Live*'s "Coffee Talk with Linda Richman," this entertaining idea still requires much more to bridge the chasm in understanding.

Like other herpesviruses, HHV-8 exhibits a lytic phase following primary infection, enabling further infection of host cells, followed by viral latency that is thought to provide a means for surviving immune-mediated destruction. If vGPCR is expressed in only a small percentage of HHV-8–infected cells, how is this signaling network sustained? Is vGPCR expressed chronically at low levels or periodically with burst-type elaboration? Because so few KS cells express vGPCR, this factor would be a poor choice as a therapeutic target. HIV Tat induces both vGPCR activity and expression of cytokines involved in development of KS.[4] Although use of combined antiretroviral therapy has reduced the incidence and severity of KS, durable complete remissions of aggressive disease are rarely observed.

With activation of many different pathways by vGPCR, can inhibition of VEGF expression result in KS remission? It is hoped that a planned National Cancer Institute (NCI)–supported AIDS Malignancy Consortium (AMC) trial of veglin, a novel antisense oligonucleotide reagent that targets all forms of VEGF, will answer this question. Use of bortezomib to target nuclear factor–κB (NF-κB), which is involved in HHV-8 and HIV pathogenesis, could treat both KS and AIDS. Because c-Kit and platelet-derived growth factor (PDGF) expression is increased in KS, a pilot study of imatinib was undertaken.[5] Based on the success of this study, an AMC phase 2 trial of imatinib for KS will begin soon.

Members of the ephrin B/EphB family have been shown to play a role in growth and invasiveness of a variety of tumors.[6] Ephrin B2 is a ligand that reverse-signals inside of the cell on which it is expressed, after binding with any of the 3 EphB receptors. Presently, we know nothing about the EphB receptor (or its cell source) that signals through ephrin B2 to maintain KS cell survival. Does ephrin B2

have any other role in KS other than antiapoptosis? Given the burgeoning importance of ephrin B/EphB in cancer, it will be only a matter of time before testing of their antagonists begins. With these and other options to molecularly target KS, it is very possible that we will be able to outsmart KSHV/HHV-8. If so, this would be a first for any of the known human tumor viruses. ■

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GENE THERAPY

Comment on Neff et al, page 997

Selection without harm: drug resistance gene therapy hits the big time

Stanton L. Gerson CASE WESTERN RESERVE UNIVERSITY

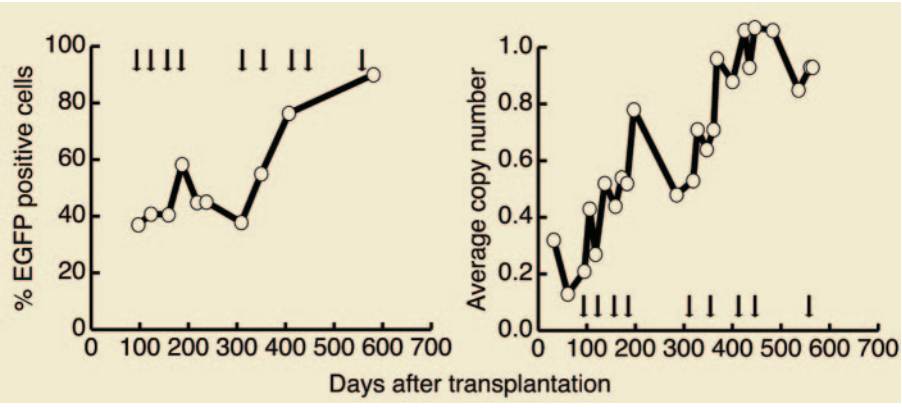
In this issue, Neff and colleagues provide an important advance in the field of hematopoietic stem cell gene therapy: the ability to select for transduced progenitor cells over time in a large animal.

The drug selection gene used is methylguanine-DNA methyltransferase (*MGMT*). Originally, this gene was identified as a DNA repair gene that encodes a protein that acts solo and in a suicide manner. The MGMT protein repairs DNA adducts at the O^6 position of guanine, a favored cytotoxic lesion induced by the chloroethylating nitrosoureas and methylating agents such as temozolomide. Both agents have since been shown to be stem cell toxins, making them ideal drugs for stem cell selection. To optimize the degree of resistance, the authors employed a mutant form of the *MGMT* gene, encoding a protein resistant to a potent inhibitor now in clinical trials, O^6-benzylguanine.

The authors transduced purified hematopoietic stem cells from 2 dogs with retrovirus containing the mutant *MGMT*(P140K) gene and infused the autologous cells back into the dogs after lethal irradiation. Thereafter, the dogs were treated over the course of more than a year with increasing doses of temozolomide. Over time, hematopoiesis was replaced by progeny of the transduced cells, blood counts remained stable despite increasing doses of temozolomide, and there was no evidence of marrow failure or myelodysplasia or leukemia. Analysis of insertions indicated polyclonality of the selected cells, with common insertions observed in both myeloid and lymphoid cells. A very high degree of gene transfer (over 90% with an estimated copy number approaching 1 at the end of the experiment) was observed.

While this has technically been done before in mice by a number of investigators,[1,2] including the more convincing validation of selection at the level of the repopulating stem cell after secondary transplantation,[3] these investigators are the first to address the issue in a large animal, where more stem and progenitor cells participate in hematopoietic reconstitution, where there is the potential for stem cell failure from "overselection," and where longitudinal follow-up provides ample time for insertional mutagenesis and leukemic transformation. Previous studies in mice have shown a remarkable degree of selection, more than 500-fold at the level of the repopulating stem cell.[3] Together, these studies suggest that after transplantation of mutant *MGMT*–transduced stem cells, patients will tolerate a strong degree of stem cell selection without prior myeloablation, a safer, perhaps outpatient, procedure. Furthermore, where complete stem cell replacement is not needed, drug treatment for selection could be tailored to the needed engraftment level.

Since no long-term adverse sequelae were noted by Neff and colleagues, albeit only with follow-up of slightly more than one year, these data support ongoing efforts to begin human clinical trials transducing stem cells with the *MGMT*(P140K) drug selection gene by retroviral and, eventually, lentiviral gene transfer for cancer and perhaps nonmalignant conditions. ■



In vivo selection in dog G179 after multiple cycles of O^6BG and temozolomide. See the complete figure in the article beginning on page 997.

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