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Research Focus

Interference with HH–GLI signaling inhibits prostate cancer

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The Hedgehog–Gli (Hh–Gli) signaling pathway controls many aspects of tissue patterning, cell proliferation, differentiation and regeneration and regulates cell number in various organs. In adults, the Hh–Gli pathway remains active in a number of stem cells and regenerating tissues. Inappropriate and uncontrolled HH–GLI pathway activation has been demonstrated in a variety of human cancers. Three recent papers show that components of the pathway are expressed in human prostate tumors and, more importantly, that prostate cancers depend on sustained HH–GLI signaling. These data raise the possibility of a new therapeutic approach to treat this often lethal disease.

Keeping form and cell number in development, homeostasis and repair

Adult organisms, unlike embryos, largely maintain their size and shape through time. How this is achieved and how form is maintained in systems where there is continuous cell replacement is not clear. For example, epithelia from the skin and intestines shed millions of cells every day and yet the overall shape and topographic details (e.g. fingerprints) are maintained. Other changes are restricted to specific periods in adult life. For example, the mammalian female's breast tissue enlarges during pregnancy and regresses after the feeding period ends. In this case, the growth of the tissue is under hormonal control, but how the organ grows and regains the original shape is unclear. Extreme examples of this process can be found in the regenerative capacity of salamanders. Newts can regenerate whole limbs, tails, jaws, lenses and even neural tissue throughout life. This seems to be the result of the plasticity and local reprogramming of differentiated cells [1].

Mammals have limited regenerative capacity and do not regenerate lost limbs. However, they have stem cells that are multipotent and can contribute to tissue and organ repair and homeostasis. Many human adult organs and tissues, including the skin, brain and blood, contain stem cells, which can self renew and give rise to progeny with various differentiation phenotypes and participate in homeostasis and maintaining cell number. Moreover, stem cells can also participate in regenerative processes. These cells therefore have great potential, and understanding their biology might lead to insights into how body-shape forms and how it is maintained. Moreover, it might lead to a better understanding and treatment of diseases involving inappropriate changes in cell number and thus form. Importantly, such diseases not only include those that are caused by cell loss, such as degenerative diseases (e.g. Parkinson's disease), but also those that are caused by non-homeostatic increases in cell number; that is, cancer.

HEDGEHOG–GLI signaling in the control of stem-cell behavior and cancer

Work during the last two decades has shown that a handful of phylogenetically conserved signaling pathways are crucial for embryonic pattern formation and in stem-cell renewal and regeneration. These include the fibroblast growth factor (FGF), epidermal growth factor (EGF), WNT and HEDGEHOG (HH) intercellular signaling pathways. Of these, HH signaling has been discovered in a surprising number of sporadic human cancers [2], including basal-cell carcinoma, medulloblastoma and one of the most common cancers in men, which is lethal in advances stages: prostate cancer [3–5].

In humans, three HH genes have been isolated: *SONIC* (*SHH*), *INDIAN* (*IHH*) and *DESERT* (*DHH*) *HEDGEHOGS*. They produce secreted glycoproteins that act through several components, including the transmembrane receptors PATCHED 1 (PTCH1) and SMOOTHENED (SMOH), to initiate a complex intracellular signaling cascade that ultimately leads to the activation of the GLI zinc-finger transcription factors (Figure 1). There are three GLI proteins, GLI1–3, which act in a context-dependent combinatorial manner. GLI1 and GLI2 often have a positive action, whereas GLI3 is frequently a negative regulator of HH signaling. Studies in model systems have shown that the Hh–Gli pathway controls the normal development and growth of several organs, including the skin, brain, pancreas, gut and prostate, at different stages of development [6,7]. Furthermore, it has been shown to control the number of embryonic and postnatal cells with stem-cell properties in different niches in the brain [8].

It is widely thought that tumors arise and grow as a result of alterations in the behavior of stem cells or their early descendants and of inappropriate expansion of these multipotential lineages. Evidence for the participation of stem cells in cancer derives from teratocarcinomas. These tumor cells can act as stem cells by participating in organogenesis after implantation in mouse blastocysts [9].

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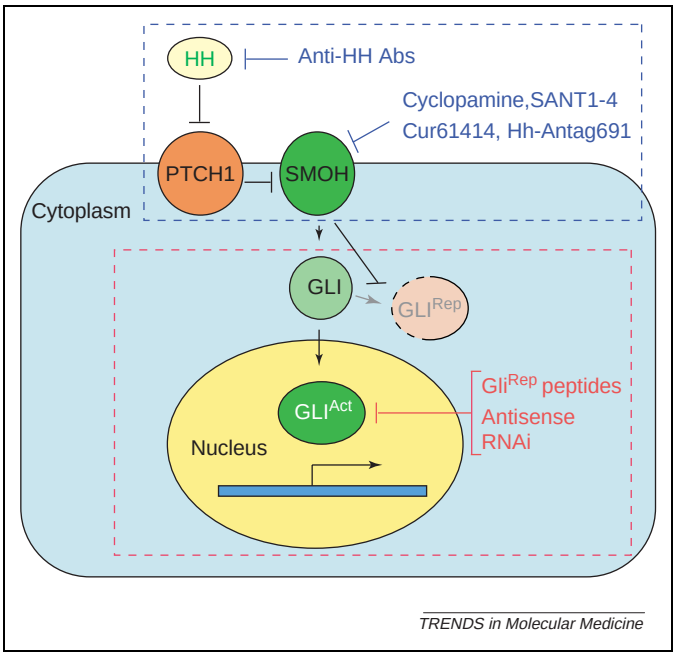

Figure 1. Schematic representation of the HH–GLI signaling pathway. A diagram of the subcellular location of essential components of the pathway. Note that SMOH might function to both potentiate the formation of GLI activators and repress the formation of C-terminally deleted repressor forms. The figure indicates possible therapeutic agents acting extracellularly on the HH ligand (anti-HH antibodies), at the membrane level by blocking Smoothened (SMOH; dark green) (cyclopamine, SANT1–4, Cur61414 and Hh-Antag691) or at the nuclear level by directly blocking the positive GLI action (red text). Abbreviations: HH, Hedgehog; PTCH1, Patched 1; GLI^{act}, GLI activator; GLI^{Rep}, GLI repressor forms (mostly those of GLI3).

The concept of 'cancer stem cells' also derives from leukemias, in which the disease can be transplanted by the grafting of a single cancer stem cell [10]. Evidence for the existence of cancer stem cells in solid tumors is still incomplete: rare cells in brain [11,12] and breast cancers [13] have been proposed to act as cancer stem cells. Together, these studies raise the question of the mode of action of HH–GLI signaling in the normal prostate, in putative prostate stem cells and in prostate cancer.

Hedgehog–Gli signaling in prostate development

The development of the mouse prostate requires Hh signaling. Although the initial formation of prostate buds does not require Hh function, Shh is necessary for maintaining appropriate prostate growth, proliferation and tissue polarity [14]. In the developing rat prostate, *Shh* is expressed by epithelial cells, acting on nearby $Ptch1^{+}Gli1^{+}$ stromal cells [7]. The HH–GLI pathway is also active in the adult human prostate, but the expression of *SHH*, *PTCH1* and *GLI1* appears to be confined to the epithelial cells of the normal prostate gland [3]. The results of an initial study suggesting that *GLI1* is only expressed in the prostate stroma [15] have not been reproduced [3]. Whether the differences obtained in the rodent and human studies represent species-specific changes or variability between adult and embryonic tissues remains unclear.

HEDGEHOG–GLI signaling in human prostate cancer

HH–GLI pathway components are also expressed in prostate cancers of different grades and locations. *In situ* lesions show the expression of *GLI1*, *SHH* and *PTCH1*, with the expression of *GLI1* and *PTCH1* serving as readouts of an active HH–GLI pathway [3]. The level of expression within tumors is variable, but in several cases the expression of *SHH* and *GLI* is upregulated compared with normal prostate epithelial tissue (Figure 2b) [3]. Indeed, high SHH protein levels correlate with cell proliferation and tumor presence, suggesting a potential use for SHH as a marker for prostate tumorigenesis [3]. Two other studies report that the HH pathway is activated at higher levels in fresh samples of metastatic lesions [4] and in advanced prostate cancers [5], in which activation is associated with elevated *SHH* expression and/or loss-of-function mutations of *SU*(*FU*), which is a negative regulator of HH–GLI activity [5]. Metastatic cells might acquire a selective advantage that enables them to leave the prostate epithelial niche as a result of potent cell-intrinsic activation of GLI signaling [3] and/or the acquisition of higher levels of receptor-mediated HH signaling [4] (Figure 2a). Consistent with these findings, exogenous Shh increases the growth of prostate cancer cell-line xenografts [15].

Evidence that interference with HEDGEHOG–GLI signaling inhibits prostate cancer

Importantly, the use of HH–GLI pathway antagonists has demonstrated the requirement of HH–GLI signaling for the sustained growth of a variety of human cancers, such as medulloblastoma, basal-cell carcinoma, small-cell lung cancer and carcinomas of the pancreas, esophagus and stomach, among others [2,16].

Three recent reports show that the growth of prostate cancers can be inhibited using specific HH antagonists that block the pathway at three different levels (Figure 1): (i) anti-HH antibodies have proven useful to block the proliferation of primary prostate tumors and cell lines [3,4], suggesting that a percentage of prostate cancers require continuous ligand–receptor activity for growth; (ii) cyclopamine, a small-molecule plant alkaloid [17] that antagonizes SMOH function [18], inhibits the growth of prostate cell lines [3–5] and primary prostate tumors [3,4], in addition to subcutaneous xenografts [4]. The treatment of prostate cancer cell lines with cyclopamine has also been shown to reduce cell invasiveness [5]; and (iii) specific small-interfering RNAs (siRNAs) against GLI1, which is the final and essential element of the HH–GLI pathway, inhibit the growth of metastatic prostate tumor cell lines [3]. These results are encouraging and suggest the possibility of using SHH, SMOH and, in particular, GLI1 as anti-cancer therapeutic targets for prostate cancer, in addition to other tumors of any grade.

Interference with Hedgehog–Gli signaling in other cancer models

The potential success of therapeutic approaches inhibiting the activity of the HH–GLI pathway to treat human cancers is further suggested by two recent *in vivo* studies. The systemic treatment of $Ptch1^{+/-}p53^{-/-}$ medulloblastoma-carrying mice with cyclopamine [19] or the antagonist Hh-Antag691, which also blocks the function of Smo [20], results in an inhibition of tumor growth,

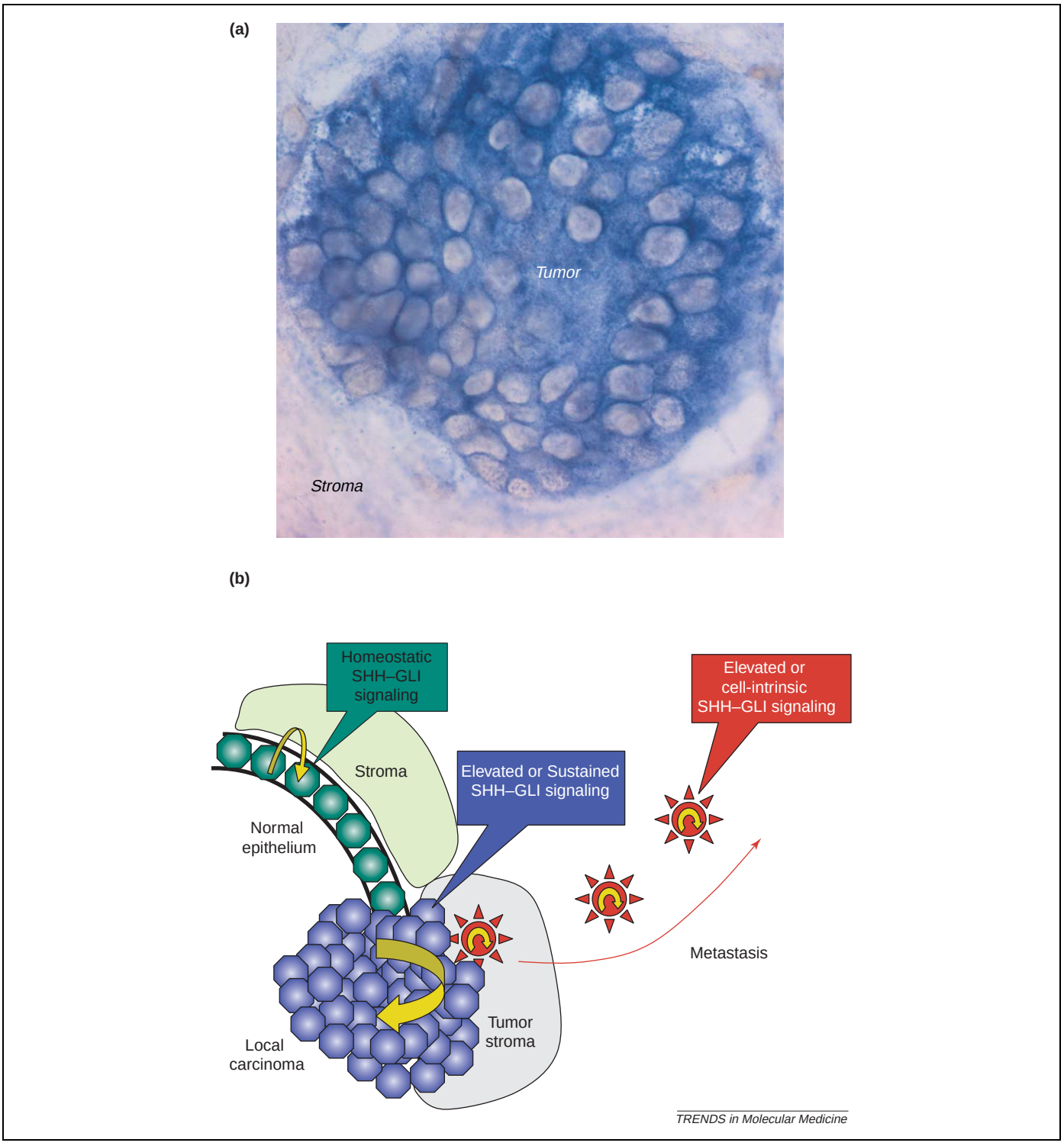

Figure 2. The development of prostate cancer from the normal epithelium, through a local carcinoma and during metastasis, correlating with the deduced activity of the HH–GLI pathway. **(a)** The expression of *SHH* mRNA in human prostate carcinoma detected by *in situ* hybridization (blue color) [3]. The surrounding stroma is negative. **(b)** The expression of *SHH*, *PTCH1* and *GLI1* in adult human prostate tissue is detected in the epithelium and not the stroma [3], unlike in the developing rat prostate, in which *Ptc1* and *Gli1* are expressed in the stroma [7]. Signaling is proposed to occur within the epithelium in adult men. The role of the stroma thus remains unclear in the context of SHH–GLI signaling and prostate cancer. Increases in SHH–GLI signaling in local tumors and metastases in relation to the normal epithelium are indicated by yellow arrows. Metastases might derive from cell intrinsic or ligand-dependent increases in GLI function. The red arrow depicts the departure of metastatic cells from the site of tumor initiation.

improving the health of treated mice. Carrier-only treated mice developed full-blown medulloblastoma. Systemic treatment *in vivo* thus appears to have beneficial effects. Similarly, oral cyclopamine treatment of Ptch1$^{+/-}$ mice prevented basal-cell carcinoma development after UV irradiation [21]. Moreover, the topical treatment of basal-cell carcinoma lesions with cyclopamine has been reported to produce a beneficial effect in patients [22].

A related study also shows that basal-cell carcinomas induced by the conditional expression of *Gli2* in transgenic

mice regress following inactivation of the transgene [23]. This is consistent with previous studies of conditional oncogenes in mice, but also shows that several non-tumorigenic cells remain in the skin, which appear to be 'sensitized' so that reactivation of the transgene leads to a more aggressive tumor [23]. It is unclear if such dormant cells represent 'primed' stem cells that persist, raising the possibility that some cells might escape anti-HH–GLI therapeutic intervention. Alternatively, the tumor stroma might remain modified after tumor regression, enabling the maintenance of dormant cancer stem cells or normal cells to gain an aggressive behavior early after transgene reactivation because these would find an already appropriate environment. However, it should be noted that the transgenic mouse is radically distinct to human sporadic cancers: in these mice, all basal skin cells express *Gli2*, whether they form tumors or not, whereas in humans, only tumor cells are thought to have an active pathway.

Concluding remarks

The findings summarized here refocus our attention on the question of stem cells and cancer, which is viewed as a disease of patterning affecting stem-cell lineages. Indeed, HH–GLI signaling can affect several stem-cell lineages, such as those in the brain (e.g. [8]), but it is not yet known whether it acts on prostate stem cells, which have been proposed, but not proven, to exist in the normal epithelium [24,25]. Stem or progenitor cells could, therefore, participate in the normal homeostasis of the human prostate and it is interesting that the HH–GLI pathway is also involved in the regeneration of the mouse prostate: HH–GLI-pathway blockade with cyclopamine or anti-Shh antibodies inhibits prostate regeneration [4]. HH–GLI signaling might have a general role in regenerative processes, possibly affecting stem cells or cells that acquire stem-cell properties. In this sense, it is intriguing that experiments on urodele limb and lens regeneration show that a Hh family member is expressed in de-differentiated cells following injury [26,27].

The three recent studies [3–5] test and confirm the hypothesis of the involvement of HH–GLI function in human prostate cancer [28]. The demonstration of the crucial involvement of HH–GLI signaling in prostate cancer, however, was largely unexpected within the prostate cancer field. Indeed, recent reviews do not mention any element of the HH–GLI pathway (e.g. [29]) and extensive microarray data failed to uncover them (e.g. [30]).

Thus, the new data call for a reassessment of the focus on research on prostate cancer and provide a clear rationale for therapy, starting with advanced, presently untreatable, diseases. These results also suggest the need for additional studies (Box 1), including work with primary human tumors, transgenic and sporadic mouse cancer models and toxicology assays. This will hopefully lead to the rapid patient testing of cyclopamine, a natural drug that targets SMOH, whereas the more promising anti-GLI siRNA technology awaits efficient *in vivo* delivery methods.

Box 1. Outstanding questions

What is the normal role of SHH–GLI signaling in the adult prostate?
Are there prostate stem cells? Is their behavior regulated by SHH–GLI signaling?
Are there cancer stem cells in prostate tumors?
What is the role of the stroma?
Are increased levels of GLI function the universal determinant for tumor initiation and metastases?
Is SHH–GLI function required for all prostate tumors of all grades?
How do the multiple genes involved in prostate cancer initiation and progression feed into or interact with SHH–GLI function?
Is the inhibition of SHH–GLI function sufficient to kill all cells within a tumor?
What are the side effects of local or systemic interference with SHH–GLI signaling in patients?
Can cyclopamine be produced as an effective therapeutic?
How can RNA-interference technology be vectorized for use *in vivo*?

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CCL3L1 dose and HIV-1 susceptibility

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Several genetic factors influence HIV-1 susceptibility or AIDS disease progression. A recent study reported on what could be a particularly important genetic determinant for HIV-1 susceptibility and disease progression: copy number of a chemokine gene termed *CCL3L1*. Individuals with low copy numbers of the gene, relative to their ethnic background, were associated with markedly enhanced HIV-1/AIDS susceptibility. These findings define an important new genetic determinant of HIV-1 susceptibility and further emphasize the importance of the chemokine system, either as elements that inhibit HIV-1 infection or that modulate antiviral immune responses.

Introduction

HIV-1 has infected many millions of people worldwide and has become one of the most serious health problems of modern times. More resources have been directed to the understanding of HIV-1 infection and AIDS pathogenesis than to any other infectious agent in human history. During the past ten years, a better understanding of HIV-1 has emerged with the discovery that several chemokine receptors function as HIV-1 co-receptors and facilitate HIV-1 entry into $CD4^+$ cells. CCR5 and CXCR4 are the most important co-receptors for HIV-1 transmission and AIDS pathogenesis. Strains of HIV-1 that use CCR5 (R5 viruses) are preferentially transmitted and found during the early stages of infection, whereas viruses that use CXCR4 (X4 viruses) are associated more with later-stage disease. The importance of the chemokine receptor CCR5 as a co-receptor that facilitates viral entry into cells was highlighted by the identification of the CCR5 Δ32 mutation; individuals homozygous for this allele (~2% of Caucasians) lack cell-surface expression of CCR5 and are highly resistant to the acquisition of HIV-1 [1]. However, a number of other genetic polymorphisms in the chemokine system have since emerged that also influence HIV-1 susceptibility or disease progression (Box 1) [2,3].

Box 1. The contribution of chemokine or chemokine receptor polymorphisms to HIV-1/AIDS susceptibility

CCR5 Δ32: CCR5 Δ32 homozygotes are resistant to HIV infection; heterozygotes show slower disease progression.
Other *CCR5* polymorphisms: numerous polymorphisms in *CCR5*, particularly promoter regions, affect CCR5 expression and the rate of progression to AIDS.
CCR2–64I polymorphism: associated with slower progression to AIDS.
CX_3CR1: rapid progression to AIDS in HIV-1-infected individuals who are homozygous for a variant of CX_3CR1. Two amino acid changes result in markedly impaired binding of CX_3CR1 to its ligand CX_3CL1.
CXCL12 (SDF-1): individuals who are homozygous for *SDF1–3′A* show a delayed onset of AIDS.
CCL2 (MCP-1): the MCP-1 −2578G allele is associated with a 50% reduction in the risk of acquiring HIV-1.
CCL5 (RANTES): the In1.1C allele is associated with a decreased expression of CCL5 and rapid progression to AIDS.
CCL3L1: low *CCL3L1* gene copy numbers, relative to the ethnic population average, is associated with markedly enhanced HIV-1 susceptibility and progression to AIDS.

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