Hypoxic homing in prostate cancer

By Tracey Baas, Senior Editor

A U.K. team has engineered an oncolytic virus with multiple regulatory elements that target it specifically to hypoxic prostate cancer cells.¹ Delivery of these viruses was effective in mice with human prostate tumors, although it remains unclear whether relying on hypoxic regulation as the only homing determinant will be sufficient in metastatic tumors, which are much smaller than solid tumors and not overtly hypoxic.

The group's delivery vehicles of choice for the oncolytic virus were macrophages, which naturally home to hypoxic areas and respond to low oxygen levels by upregulating hypoxia-inducible transcription factors.

Hypoxic areas are not exclusive to tumors and also occur in wounds, arthritic joints and atherosclerotic plaques. Thus, the researchers constructed an oncolytic adenovirus with a hypoxic promoter element that activates viral replication in the presence of hypoxia-inducible transcription factors and a recombinant prostate tumor regulatory sequence that activates replication in the presence of prostate-specific proteins (see **Figure 1, "Oncolytic control"**).

In immune-compromised mice with human prostate tumors, transduced murine macrophages migrated into the hypoxic tumor regions and released large quantities of oncolytic virus, which then killed tumor cells and increased survival.

The transduced macrophages did not promote the formation of new blood vessels, and tumors in the treated mice were less vascularized than tumors that received nontransduced, control macrophages.

Results were published in *Cancer Research*, and the team was co-led by Norman Maitland and Claire Lewis. Maitland is a professor of molecular biology at **The University of York**, and Lewis is a professor of molecular and cellular pathology at **The University of Sheffield**.

The team also included researchers from the **University of Oxford**, **University of Liverpool**, **University of Kent**, **Sheffield Hallam University**, **University of Leicester**, **Mount Vernon Hospital** and **Uppsala University**.

Lewis noted that the macrophage-adenoviral system could potentially deliver therapeutic genes to other types of tumors or diseased tissues that contain hypoxic sites, like arthritic joints, myocardial infarcts and atherosclerotic plaques.

For other types of tumors, the recombinant prostate tumor regulatory sequence could be replaced by an appropriate tumor-specific regulatory sequence, she said. For nonmalignant diseases, the oncolytic virus would be replaced by a replication-deficient adenovirus in which

Figure 1. Oncolytic control. Macrophages are first transfected with a vector (light grey rectangle) in which the gene encoding the adenovirus small early region 1A (E1A) protein (yellow oval) is under the control of a hypoxic response element (HRE). They are then co-transduced with a conditionally competent, oncolytic adenovirus (Ad; dark grey rectangle)—the replication of which can be regulated by either E1A protein or a regulatory sequence called PPT. PPT is a composite of promoter enhancers from three genes expressed in prostate tumor cells: *TCR- γ alternate reading frame protein (TARP)*, *prostate-specific antigen (KLK3; PSA)* and *prostate-specific membrane antigen (PSMA; FOLH1; GCPII)*.

In regions of low oxygen, macrophages upregulate a transcription factor called hypoxia-inducible factor 1 (HIF1; light blue oval) which binds the HRE to produce E1A protein [a]. Production of E1A (yellow oval) then initiates adenovirus replication in the macrophage, leading to virus production [b].

The virus is then released and can enter surrounding cells. When the virus enters normal cells, lack of either E1A or the prostate-specific factors that stimulate the PPT sequence prevents further viral replication [c]. When the virus enters a prostate cancer cell, the PPT sequence is activated (dark green oval), leading to a further round of replication of the oncolytic virus and then cell death [d].

In regions of normal oxygen, macrophages do not upregulate HIFs. As a result, E1A and adenovirus production do not occur.

a therapeutic gene was placed under the control of a tissue-specific regulatory sequence.

Nancy Boudreau, professor of surgery at the **University of California, San Francisco**, also thinks other regulatory sequences could be

explored but said the first focus should be on using something other than hypoxia to amplify the virus in macrophages.

“Not all tumors may be overtly hypoxic, particularly smaller but potentially aggressive metastatic tumors, so relying on hypoxia to improve specificity and activation of the virus may be limiting,” she said. “Other factors that contribute to homing of macrophages to tumors could be exploited. One example is secretion of specific breast cancer chemokines that attract macrophages to the breast tumors. They could modify their system to allow a chemokine or some other secreted protein to induce viral replication in the macrophage.”

Look before you leap

The authors acknowledged that there are limitations to using immune-compromised mouse models because the host immune system can affect tumor progression. Thus, the researchers plan to adapt their system to replace adenovirus with viruses that infect mice, like lentivirus or vaccinia virus, and to use normal, immune-competent mice that express human genes.

Simon Barry, head of the vascular modulation group at **AstraZeneca plc**, wanted to see how delivery differs in immune-competent mice and also was interested in how tightly controlled the viral regulation is in nonhypoxic tissues or in noncancerous prostate tissues.

Because prostate cancer tends to occur in older patients, there often are comorbidities like arthritis with hypoxic tissues in the joints or atherosclerosis with hypoxic tissues in the vascular system. Thus, said Barry, it would be good “to see studies done in mouse or rat models of atherosclerosis or lung fibrosis—to get a glimpse of situations where there are comorbidities.”

Lewis doesn't think effects in off-target hypoxic tissue will be an issue. She noted that viral replication remains in the macrophage until the cells are in the presence of prostate-specific proteins.

Stuart Naylor, CSO of therapeutic vaccine company **Oxford BioMedica plc**, thinks the approach's requirement of *ex vivo* manipulation of macrophages may prove cumbersome. “The requirement to both transfect macrophages with a vector and then transduce them with an adenovirus may be challenging to validate prior to clinical evaluation,” he said.

Barry agreed. “To routinely transfect macrophages with a high probability of success and maintain viral expression would be a significant hurdle,” he told *SciBX*. “To use this therapy in the clinic, you would also have to be in a position to generate the ‘killer’ macrophages for each individual patient.”

The work is not patented and not available for licensing.

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