Tissue factor-activated prodrugs

By Lauren Martz, Staff Writer

A team at **The Scripps Research Institute** has designed a prodrug of doxorubicin that is activated by components of the tissue factor coagulation cascade present in high concentrations in the tumor microenvironment, thus avoiding off-target effects.¹ The prodrugs, which are licensed to **Affinity Pharmaceuticals Inc.**, showed better efficacy against primary tumors and metastases than standard doxorubicin in mice.

Tissue factor is a glycoprotein that has been associated with many aggressive cancers,² where high levels on the tumor cell surface can trigger oncogenic proliferation, angiogenesis and metastasis.³

However, tissue factor's normal function of starting the coagulation cascade when bound to factor VIIa has made inhibiting the protein a risky proposition in cancer because of the potential for bleeding side effects.

"In blocking tissue factor, you are blocking the extrinsic coagulation cascade, and you increase the chance of bleeding events," noted Wei-Qun Ding, assistant professor of pathology and a member of the Peggy

and Charles Stephenson Oklahoma Cancer Center at the **University of Oklahoma Health Sciences Center**.

A Scripps team led by Cheng Liu decided to take a different tack. Rather than directly targeting tissue factor in cancer, the researchers opted to use the high levels of tissue factor in the tumor microenvironment as a way to selectively activate a chemotherapy prodrug. Liu is an assistant professor of immunology at Scripps.

Liu's team developed two prodrugs of doxorubicin that were activated by the complex of tissue factor and factor VIIa or by thrombin, a downstream component of the coagulation cascade (see **Figure 1**, "Tissue factor-activated coagulation cascade").

The prodrugs were developed by attaching a small peptide to standard doxorubicin, rendering the chemotherapeutic agent inactive until the peptide is cleaved by tissue factor/factor VIIa or thrombin. One prodrug was designed to have increased solubility by appending a polyethylene glycol or succinyl substituent, while the other contained an ϵ -maleimidocaproic acid moiety that binds to albumin to prolong half-life.

In a mouse model of breast cancer, both prodrugs led to accumulation of active doxorubicin at tumors, reduced tumor volume and increased survival in comparison with standard doxorubicin.

In a human breast cancer xenograft model, the albumin-binding prodrug completely eradicated tumors without recurrence, and in a model of breast cancer metastasis to the lung, this prodrug regressed established metastases.

The prodrugs were not activated in the liver and other tissues and had less toxicity-associated weight loss than standard doxorubicin.

The findings were published in *Cancer Research*. The paper also included a researcher from the **University of North Carolina at Chapel Hill**.

A safer alternative

Liu told *SciBX* the next step for the work will be to select a lead compound. Affinity did not provide details about their plans with these compounds.

Figure 1. Tissue factor-activated coagulation cascade.

The tissue factor (TF)-induced coagulation cascade is constantly activated in the tumor microenvironment because TF is overexpressed on tumor cell surfaces and inactive coagulation factors, including factors V (FV) and X (FX) (light blue circles), move out of the excessively permeable tumor vasculature.

The coagulation cascade is activated when TF forms a complex with factor VII (FVII), which is activated to FVIIa. The complex then induces a series of reactions that activate coagulation factor enzymes (dark blue circles), ultimately converting prothrombin to thrombin and fibrinogen to fibrin, activating blood clotting.

Liu *et al.* designed doxorubicin prodrugs that exploit the tumor-specific TF activation. The prodrugs are separately hydrolyzed by the TF:FVIIa complex and by thrombin in the tumor microenvironment, but not in the microenvironment of healthy tissue. This results in accumulation of doxorubicin in the tumor.

Ding thinks the prodrug technology is flexible in terms of both the drugs involved and the indications that could be pursued.

“From a scientific point of view, I expect that this could be applied to many different chemotherapeutics, such as paclitaxel and cisplatin, to

make them more active specifically against the cancer,” he said. “The study also models breast cancer, but there could be many types of cancers that could be treated with this kind of strategy, as tissue factor is expressed by many types of cancer cells.”

“Tissue factor overexpression is widely reported in cancers such as pancreatic and gastric cancers. The most interesting example will be glioblastoma multiforme, which has very high tissue factor expression and represents an

unmet medical need,” noted Liu.

Paul Parren, SVP and scientific director of **Genmab A/S**, wanted to see more data on what happens to healthy cells that express tissue factor. “Doxorubicin-induced cell death may also happen at sites where non-tumor cells express tissue factor,” he said. “The impact in terms of affecting coagulation or causing damage to normal tissues is not known, and this area needs further investigation.”

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University of Oklahoma
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Ding agreed that “it is always possible that the drug is activated at other sites if the patient has an injury or thrombosis. You need to be careful if the patients have a high tendency for thrombosis or injury. This could be dangerous depending on the type of tissue with the damage. For example, chemotherapeutic accumulation at an injury in the heart or the brain could be particularly dangerous and could result in damage to those tissues.”

He did note that “these drugs are normally delivered systemically with the same risk factors.”

Liu told *SciBX* that Scripps has filed an application for a patent and it has been licensed to Affinity Pharmaceuticals.

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COMPANIES & INSTITUTIONS MENTIONED

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