Paradoxical P2X7

By Tracey Baas, Senior Editor

Italian researchers have shown *in vivo* that inhibiting the P2X7 receptor, rather than agonizing it as previously thought, can treat cancer.[1] The findings could open up a new disease area for companies developing P2X7 antagonists to treat pain, multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis.

P2X and P2Y receptors collectively are responsible for mediating cellular responses to extracellular ATP. Multiple groups have identified five P2Y and two P2X receptors in a variety of cancers.[2] The teams have shown that growth of the malignant cells were kept in check by ATP agonizing purinergic receptor P2Y G protein–coupled 1 (P2RY1; P2Y1) and P2Y2 (P2RY2) to slow cell proliferation and purinergic receptor P2X ligand-gated ion channel 5 (P2RX5; P2X5) and P2Y11 (P2RY11) to inhibit cell differentiation. In the same way, growth was checked by ATP agonizing P2X7 (P2RX7) to induce cell death.

However, two studies published in 2005 and 2009 led by Francesco Di Virgilio showed that P2X7-overexpressing cell lines were resistant to chemically triggered apoptosis and had the hallmarks of tumor transformation, including increased mitochondrial potential, synthesis of ATP and serum-free proliferation.[3,4] Di Virgilio is a professor of clinical pathology and chairman of the Department of Experimental and Diagnostic Medicine at the **University of Ferrara**.

Inhibiting P2X7 blocked all of those hallmarks. Thus, the Italian group hypothesized that the receptor might have roles in both cell survival and cell death in cancer. Cell survival would be reliant on conditions of normal physiological levels of extracellular ATP, whereas cell death would be reliant on conditions of high pharmacological levels of ATP.

Now, Di Virgilio's group has tested this hypothesis *in vivo*. Immunodeficient mice inoculated with P2X7-overexpressing human cells generated tumors much faster than mice given human cells with normal P2X7 expression. In immunocompetent mice, inoculation with murine colon carcinoma cells that had high P2X7 expression led to much faster tumor formation than inoculation with carcinoma cells that had low P2X7 expression.

In both models, cells with high levels of P2X7 showed greater expression of VEGF and vascularization than cells that had normal levels of P2X7.

In mice inoculated with mouse melanoma or human neuroblastoma cells, P2X7 blockade with the selective P2X7 antagonist AZ10606120 or P2X7 knockdown with shRNA decreased tumor growth compared with no treatment or normal P2X7 expression, respectively.

The results were published in *Cancer Research*.

According to Di Virgilio, P2X7 antagonists have not been extensively studied in cancer because the target "is better known as a cytotoxic rather than a growth-promoting receptor. Thus, people believe that P2X7 agonists should be used to treat cancer, rather than antagonists."

"I would be interested to see further animal work from Di Virgilio's team that would provide the kinetics to show if the P2X7 antagonists reach all parts of the tumors without inducing systemic toxicity or inflammation. They might need to develop special antagonists targeted at the tumor," said Pieter Dagnelie, associate professor of nutritional epidemiology at **Maastricht University**.

Geoffrey Burnstock, emeritus professor and president of the Autonomic Neuroscience Centre of the **University College London Medical School**, agreed. "Because P2X7 receptors are widely expressed in different tissues, P2X7 antagonists will need to be shown to be selective for tumor cells with little effect on P2X7-mediated activities in other tissues. Also, there are many human P2X7 polymorphisms that might not all respond to an antagonist."

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"There is a strong need to go beyond overexpression studies and immunostaining of P2X7 in tumor samples and rather address dependence of cancers on P2X7 for initiation, maintenance and metastasis," said Joanna Hergovich Lisztwan, research leader of oncology at **Evotec AG**. "There needs to be a deeper understanding of how the exact mechanisms involved in P2X7's regulation of mitochondrial metabolism would lend further argument to targeting this ion channel in cancer, or alternatively reveal new targets involved in the pathway."

Evotec's EVT 401, an oral small molecule P2X7 receptor antagonist, is in Phase I testing. Preclinical studies are ongoing to assess its potential in a number of indications.

Immune issues

In addition to sorting out the distribution of P2X7 receptors, another question is whether blocking the target will modulate how the immune system interacts with tumors.

For example, George Dubyak, professor of physiology and biophysics at **Case Western Reserve University**, said antagonizing the receptor "could also suppress P2X7 signaling in dendritic cells and macrophages that is essential for the antitumor immune responses elicited by several chemotherapeutic agents."

When chemotherapeutics destroy tumor cells, high levels of ATP are released from the tumor interstitial space. The ATP then acts on dendritic cell–specific P2X7 to trigger the inflammasome and production of IL-1β and IL-18, resulting in immune system–mediated eradication of cancer cells.[5]

Di Virgilio countered that his team showed that "P2X7 antagonists

caused tumor regression in immunocompetent mice. This is a first step toward showing that the immunological component might still be effective. But future studies in mice will need to include extended time courses while looking closely at immune-response components."

John Lust and Kathleen Donovan, both in the Division of Hematology at the **Mayo Clinic**, said P2X7 inhibitors might have additional upside by blocking chemotherapy-induced inflammation. "My laboratory has previously shown in samples from myeloma patients that P2X7-specific inhibitors developed by **Pfizer Inc.** can block ATP-induced IL-1β release and subsequent IL-6 production, particularly in chemotherapy-treated patients. IL-6 is a central growth factor for myeloma cells. We believe our results suggest a way to inhibit chemotherapy-induced inflammation that may be contributing to relapse seen with this disease."

Lust's team tested four P2X7 antagonists from Pfizer. Lust did not disclose any further details regarding the specific molecules or his collaboration with Pfizer.

P2X7 antagonists in development include **GlaxoSmithKline plc**'s GSK1482160, a purinergic ATP receptor antagonist that targets P2X7. The molecule is in Phase I testing to treat pain. The pharma declined to comment for this story.

Affectis Pharmaceuticals AG's AFC-5128, a brain-penetrant P2X7 antagonist, is in preclinical development to treat multiple sclerosis (MS) and neuropathic pain. The company expects to submit an IND within 12 months.

Affectis' CBO Luc St-Onge is looking forward to seeing results from the new paper extended to other mouse models of cancer, especially brain cancer.

Next steps from Di Virgilio's team include more extensive animal studies with P2X7 antagonists that have already gone through Phase I and II testing for other indications.

The work is not patented and is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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