Necuparanib Inhibits Pancreatic Cancer Progression and Invasion in a 3D Tumor and Stromal Cell Co-Culture System

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ABSTRACT

Pancreatic cancer is one of the most aggressive types of cancer, with only about 5% of patients surviving 5 years past the initial diagnosis. Despite advances with new chemotherapy combinations, several survival outcomes are still dismal and novel therapeutic agents or combinations are needed. Therefore, to both diagnose and treat the aggressive fibrotic microenvironment and restrain tumor proliferation is a current focus. Necuparanib (Momenta M202) is a heparin sulfamate that binds and inhibits multiple heparin-binding growth factors, chemokines, cell adhesion molecules. Here we explore how necuparanib affects the tumor and its microenvironment using a 3D collagen system mimicking pancreatic cancer. The system contains a co-culture of human pancreatic tumor cells and pancreatic stellate cells (PSCs), the most abundant stromal cell in the pancreas. We found that necuparanib inhibits pancreatic tumor cell growth, decreases tumor cell invasion, and permits administration of higher concentrations of gemcitabine. Here we explore how necuparanib alters the tumor microenvironment and enhance the therapeutic effect of gemcitabine, a chemotherapeutic agent, was reduced anti-tumor activity, permitting administration of higher concentrations of gemcitabine. Necuparanib in combination reducing anticoagulant activity, permitting administration of higher concentrations of gemcitabine demonstrates that necuparanib is a promising multi-targeted agent.

BACKGROUND

Necuparanib, derived from heparin, was rationally engineered to preserve/potentiate the anti-tumor activity while substantially reducing anticoagulant activity, permitting administration of higher doses relative to heparin in patients. Necuparanib in combination with gemcitabine and anti-angiogenic agents is currently being tested in a Phase 2 clinical trial in patients with metastatic pancreatic cancer (Clinical Trials Identifier: NCT01625489).

The objective of this study was to test the ability of necuparanib to reduce proliferation and invasion of pancreatic tumor cells into surrounding matrix in 3D cultures.

METHODS

3D Cell Culture Setup

- Pancreatic tumor cell lines (AsPC-1, Capan-2; red) and human pancreatic stellate cell line (green) were plated into Matrigel coated inserts of a 24-well plate. 10,000 cells were plated per well. Each well contained 0.5 ml of Matrigel during the first 4 days of culture. After 4 days, media was changed, and necuparanib or vehicle was added for 2 days. Quantitative analysis of the culture was conducted using confocal microscopy. A representative area of the culture was obtained, and circularity (ratio of circumference to area) of the spheroid in the image was calculated.

RESULTS

Necuparanib inhibits pancreatic tumor cell growth.

- Necuparanib affects tumor cell proliferation in a dose-dependent manner. AsPC-1 control versus co-culture of AsPC-1 and stellate cell in 3D culture. Tumor cells become invasive in a bud-like outgrowth into the matrix.

Necuparanib inhibits pancreatic tumor cell invasion into the surrounding matrix.

- Necuparanib localizes both intracellularly and intercellularly.

Necuparanib and gemcitabine in combination exhibit an additive effect compared to gemcitabine alone.

CONCLUSIONS

In summary, our in vitro data indicate that in this model necuparanib affects tumor cell growth, PSC growth, and tumor cell invasion likely by binding growth factor and chemokines that modulate tumor-stromal cell interactions.

- Necuparanib inhibited the growth of pancreatic tumor cells in 3D culture.
- Pancreatic tumor cells such as AsPC-1 and Capan-2 are only found to be invaded when co-cultured with pancreatic stellate cells (PSCs) in 3D culture.
- Necuparanib inhibited invasion of AsPC-1 cells while decreasing the number of PSC outgrowth in a dose-dependent manner.
- Necuparanib, enoxaparin and dalteparin inhibited invasion in a dose-dependent manner. 
- Necuparanib, derived from heparin, was rationally engineered to preserve/potentiate the anti-tumor activity while substantially reducing anticoagulant activity, permitting administration of higher doses relative to heparin in patients.

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