Student: David Jin
Title: Cytokine and Growth Factor Activation Profiling in Ascitic Fluids from Chemo-Sensitive and Resistant of Ovarian Cancer Patients
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Introduction:

Ovarian cancer is the second most common gynaecologic cancer and the leading cause of death from gynaecologic cancers. It is a difficult cancer to treat as it often goes undetected until the tumour has spread, and current treatment involves surgery to remove large tumour masses, with chemotherapy given to try and eliminate the remaining tumour cells. Although most patients respond to chemotherapy initially, the disease recurs for the majority of patients, and survival is poor.

Advanced ovarian cancer causes the accumulation of body fluid in the abdominal cavity, known as ascites. Tumour cells use ascitic fluid as a vehicle to move around in the abdominal cavity. Often, tumour cells form small clumps known as spheroids, which tend to deposit on the abdominal cavity walls and grow to form a separate tumour. This is one of the factors making ovarian cancer treatment difficult.

Recently, it has been found that the ascitic fluid taken from patients contains many cytokines and growth factors which create a favourable micro-environment in the abdominal cavity, in which the tumour cells survive and thrive. Therefore, it has been suggested that the cytokines and growth factors in the ascitic fluid play a vital role in facilitating tumour growth and spread. However, the effect of these cytokines and growth factors on ovarian cancer cells are not well understood, and this is a current area of interest in ovarian cancer research.

Furthermore, there is a huge effort underway to develop a new treatment for ovarian cancer, and a large number of new, “targeted drugs” (which target specific cell signal pathways, as opposed to chemotherapy which is toxic to all growing cells) being developed and tested, and despite a number of them showing great promise in the laboratory, few have proven effective on their own. Given the range of cytokines and growth factors found in the ascitic fluid, it is possible that the failure of these new drugs may be related to the effect of the ascitic fluid on the tumour cells.

This project is a proof-of-concept study for using ascitic fluid collected from patients with advanced ovarian cancer as an important biological sample in ovarian cancer research. The study focuses on a class of cell signal receptors known as tyrosine kinase receptors (which are the targets of a class of drugs known as tyrosine kinase inhibitors), and sets out to determine how treatment with ascitic fluid alters the activation of these receptors. The study also investigates the effects of ascitic fluid on the growth activity of cultured ovarian cancer cells. Additionally, ascitic fluid was added to an experimental drug (canertinib) which inhibits two growth factor receptors found in some ovarian cancer cells (EGFR and Her-2) to study its effects on the growth activity of cultured ovarian cancer cells treated with the drug.

Aim:

- To identify the tyrosine kinase receptor activation profile in ovarian cancer cell lines stimulated with ascitic fluid;
- To examine the growth activity of ovarian cancer cells in the presence of ascitic fluids;
- To examine the growth activity of ovarian cancer cells stimulated by ascitic fluids in the presence of an EGFR/HER-2 inhibitor;

Method:

This study used ovarian cancer cells from two established cell lines, OVCAR-5 and SK-OV-3; as well as ascitic fluid samples collected from consenting patients with advanced ovarian cancer.

For each experiment, cells taken from the cultures were grown in wells coated with a polymer to stop cells adhering to the surface of the wells, forcing the cells to form clusters, which closely emulates the behaviour of ovarian cancer cells inside the abdominal cavity of patients. The cells were grown for 5 days in a culture

medium which supports cell growth. They were then starved for 24 hours in “starvation medium”, a culture medium with only the essential nutrients for survival. The cells were then treated in the following possible conditions: (1) cells with starvation medium, (2) cells with canertinib, (3) cells with 50% patient A ascitic fluid, (4) cells with patient A ascitic fluid plus canertinib, (5) cells with 50% patients B ascitic fluid, and (6) cells with patient B ascitic fluid plus canertinib. Cells were treated with the conditions described above for 24 hours before being collected for analysis.

For experiments investigating tyrosine kinase receptor activation, the cells were broken up, and their contents (the cell lysate) were analysed using a commercial antibody array kit which detected the activated (phosphorylated) forms of a number of different tyrosine kinase receptors.

For experiments investigating growth activity, the cells were collected, treated with a dye which helps identify alive (viable) cells from dead ones, and counted on a special microscope slide (a haemocytometer) under a microscope.

Results:

1) Cells treated with ascitic fluid showed a different profile of tyrosine kinase receptor activation compared to cells not treated with ascitic fluid, and ascitic fluid from the two patients gave rise to two different activation profiles.
2) Cells treated with ascitic fluid had increased cell growth activity compared to cells which were not treated with ascitic fluid.
3) Both patients’ ascitic fluid were able to keep cells alive when treated with the experimental drug canertinib, whereas many cells not treated with ascitic fluid but treated with canertinib alone died (with clear evidence of cell death under the microscope, and reflected in the number of surviving cells).

Conclusion:

The preliminary results gathered in the course of this project suggest that cytokines and growth factors in ascitic fluid play a key role in the survival, growth, and possible increase of drug resistance of advanced ovarian cancer cells. The modest response of advanced ovarian cancer patients to EGFR/Her-2 inhibitor was well established in clinical trials, but there was no scientific rationale for that such ineffective treatment. The preliminary data from this project may shed the light on the basis of insensitivity of tyrosine kinase inhibitors in advanced ovarian cancer patients. Above all, this project has proven the concept of ascites research – it has shown that the study of ascitic fluid in ovarian cancer research is a promising area which will not only further our understanding of the biology of ovarian cancer, but also promises to reveal why so many experimental drugs have failed, and how we can overcome those barriers to develop better treatment for ovarian cancer.