**Signaling Pathways Produced By Combining DsRNA with Paclitaxal**

Introduction:

Epithelial ovarian cancer is ranked fifth among the malignant deaths of women in the United States and is considered to be more fatal than other gynecological tumors (Cannon, Santin & O’Brien, 2004). The actual cause of ovarian cancer is unknown, but one source of it can be invasive transformation of secretory cells in the fallopian tube. Ovarian cancer is classified into two categories: type I and type II. A type I tumor is when it has just begun its growth; it shows low levels of carcinomas and they are different from each other. Carcinomas are when the cancer starts to form on the lining of the internal organs and the epithelial tissue. A type II tumor has high levels of carcinomas and they are identical (Hennessy, Coleman and Markman, 2009). The most common therapy to treat ovarian cancer is cytoreductive surgery followed by a chemotherapy. Approximately 60% of patients, treated in advanced stage, have a good response to the treatment. However, 80% of the patients treated during the late stages develop recurring diseases (Di, Boer, Figdor and Torensma, 2013).

Cytoreduction surgery is when the overspread or deoxygenated tissues are removed to prevent the tumor from being resistant towards chemotherapy. There are three steps in surgery: diagnosis, determination of the stage and cytoreduction. Initially the tumor is located and then, the growth of the tumor is determined to indicate the stage. After the stage has been confirmed, the tumors that are greater than 1 cm in diameter are removed. Patients who end up with bulky residual of the tumor have a low survival rate rather than the patients with little visible residual. Surgery only improves the condition of the patient overall but does not assure survival of the patient or provide a cure for the disease (Coleman, Monk, Sood & Herzog, 2013).

In addition to a cytoreduction surgery, chemotherapy is needed to remove the residuals of the tumor. Chemotherapy is when a patient is given doses of cytotoxic drugs to kill cells. The current drugs used to treat ovarian cancer are carboplatin and paclitaxel. Paclitaxel is a mitotic inhibitor that stabilizes microtubules hence blocking the normal break down during the cell division. This inability to divide induces prolonged activation of the mitotic checkpoint causing reversion to G-phase without cell division or apoptosis. Carboplatin damages the DNA of tumor cells by crosslinking the DNA. Loss of DNA repair mechanisms in tumor cells make it unable to repair sites of carboplatin induced crosslinking. The cell is unable to replicate this DNA and stops dividing leading to tumor cell apoptosis. Other treatments involve using different drusgs like cisplatin, bevacizumab, taxane and docetaxel. Carboplatin is derived from cisplatin with very little side effects but it is lower efficiency. Bevacizumab is a drug that blocks VEGF pathway which has a critical role in cancer. Taxane and docetaxel are both in the same class as paclitaxel, all of which are derived from Taxol. Another way to improve therapeutic response is to delivers the chemotherapy directly to the tumor site by intraperitoneal chemotherapy delivery. This type of chemotherapy is given through abdominal membrane linings also known as peritoneum. Even after getting doses of chemotherapy patients have a high recurrence risk. Due to the recurrence high risk, additional treatment is needed (Coleman, Monk, Sood & Herzog, 2013).

Though there have been various advancements in the treatment of cancer there is not yet a cure . Two-thirds of the patients with advanced stage ovarian cancer tumors eventually end up getting cancer again. For platinum based drugs like carboplatin the recurrence is categorized into platinum-refractory, platinum-resistant or platinum sensitive disease. Platinum-refractory disease is when the patient fails to respond to the therapy all. Platinum-resistant disease is when cancer has recurred within six months of the treatment, while platinum-sensitive disease is when cancer has recurred after six months. Many patients end up undergoing a secondary cytoreduction or getting additional chemotherapy. After various therapies the patient may become chemoresistant to platinum and taxol based therapies and consequently the tumor eventually grows back. (Coleman, Monk, Sood & Herzog, 2013).

One novel therapy is to focus on the patient’s immune system to improve on the patient’s survival rate. From the rapid growth to the development of the bulky tumor; the disease constantly interacts with the immune system, this process is known as cancer immunoediting. There are three phases of this process: elimination, equilibrium and escape. The elimination phase describes when immune system mediates tumor cell death. The equilibrium phase is where both tumor cell death and growth is caused by the immune system, while the escape phase is when tumor cells become resistant to immune system mediated tumor cell death (Di, Boer, Figdor and Torensma, 2013).

While many therapies have been studied to improve the condition of patients, not many treatments focus on a specific patient and their immune system. This is necessary because cancer in each individual is unique and plays a slightly different role (Di, Boer, Figdor and Torensma, 2013). To prevent cancer from growing, tumor immune receptors are activated by innate immune agonists like dsRNA, making them visible targets for chemotherapy. Double stranded RNA is known to stop the immunosuppression signals caused by ovarian tumor cells and activate four innate immune receptors (Van et al., 2012). The four receptors are: TLR3 ( L. et al., 2001), RIG-I (M. et al., 2004), mda5 ( Kang et al., 2002) and PKR ( Levin and London 1978). When stimulated, these receptors activate signaling pathways which lead to proinflammatory and apoptotic responses (Van et al., 2012).

In Van et al. (2012), the effect of dsRNA on ovarian cancer cell lines was shown. There were two populations identified: dsRNA resistant and dsRNA sensitive. DsRNA resistant ovarian cancer cells do not undergo cell death when stimulated with dsRNA, while dsRNA sensitive cells die when stimulated with dsRNA. Using those two subpopulations cell death pathways activated (in dsRNA sensitive) or blocked (in dsRNA resistant), were defined. Figure 1A shows the results of dsRNA (pl:pc), carboplatin (CARBO) and paclitaxel (PAX) treated dsRNA sensitive cells both individually and combined. The columns with the stars indicate that the dual treatment caused a significant decrease in cell viability over the individual treatments. The results showed that treatments combining dsRNA with a cytotoxic agent significantly decreased cell viability. For example, dsRNA and paclitaxel combined decreased cell viability more than each treatment separately. Statistically, at four treatment concentrations, cell viability was significantly decreased with dsRNA and pactlitaxel whereas at three treatment concentrations, cell viability was significantly decreased with the combination of dsRNA and carboplatin. However, none of the treatment concentrations tested showed a significant decrease in cell viability with paclitaxel and carboplatin together.



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| Figure 1: dsRNA potentiates paclitaxel- and carboplatin-mediated cytotoxicity. A) OVCAR-3cells were treated for 48 h with paclitaxel, carboplatin, and pI:pC at the indicated concen-trations, which correspond to 0.25X, 0.5X, 1X, 2X, and 4X the ED50 concentration (left to right). Cell viability was measured via MTT assay. Values are reported relative to untreated control. Data are means (+-) of 3 independent experiments. \*P less than or equal to 0.05 for dual treatment vs. both individual treatments. B) Combination index (CI) values for each drug combination were calculated via isobologram analysis. Dot-ted line represents additivity, where CI = 1.(Reproduced from Van et al. 2012)  |

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Drug combinations can interact in three different ways: additive, antagonistic, and synergistic. Additivity is when the effect of the drug combination results in an effect that is the sum of the individual treatments. Antagonism is when two drugs combined have an effect that is less than the sum of the individual effect of treatment. Synergism is the opposite of antagonism. It is when two drugs combined have more than the additive effect of the individual treatments. Synergy is crucial to cancer chemotherapy because it is proved to be beneficial for patients. Synergy not only causes more cell death than expected by the combination of drugs, but also allows physicians to use a lower dose of chemotherapeutic drugs, often having serious side effects, while retaining high levels of tumor cell killing. If less drug can result in equivalent or more cell death when used in combination then application of drugs to a broader patient population could be possible (Chou 2006). Figure 1 B shows the combination index (CI) of the tested drug combinations at three drug concentrations; synergistic (<1), antagonistic (>1) or additive (=1) effect. The CI of paclitaxel and carboplatin suggests an antagonistic effect and dsRNA combined with either paclitaxel or carboplatin has a synergistic effect. Figure 1B concludes that dsRNA combined with paclitaxel or carboplatin induces a synergistic decrease in cell viability and can be very beneficial in developing future treatments (Van et al. 2012).

Previous studies have shown that the combination of dsRNA and paclitaxel causes synergistic cell death in ovarian cancer, however the mechanism by which this increased cell death occurs is unknown. We propose that these two therapeutic agents combined will induce overlapping signaling pathways that amplify activation of the extrinsic and intrinsic cell death cascades. For this proposal we will involve a PCR array approach to outline activated apoptotic pathways in either dsRNA or paclitaxel or the combination of dsRNA sensitive ovarian cancer cell and paclitaxel. This proposal will identify the essential pathways of the apoptotic mechanisms necessary to induce this drug combination’s synergistic effect. These essential pathways will serve as biomarkers for individual patients retaining these signaling pathways. Furthermore, these studies of mapping essential pathways to induce apoptosis will provide additional targets for further therapeutic development. In conclusion, this proposal will improve our ability to target a synergistic combination of chemotherapeutic agents to patients which are highly responsive to this treatment.

Experiment:

The goal of this experiment is to examine the mechanism of synergistic apoptosis caused by combining paclitaxel and dsRNA. This experiment will be performed by apoptosis PCR array which is mostly used for gene expression analysis. An apoptosis PCR array profiles the expression of genes which are relevant to a specific pathway or in this case the cancer. As shown in Figure 2 the PCR Array will begin by isolating RNA from the experimental samples which is done by the RNase-Free DNase Treatment. Once the RNA has been isolated, DNA is synthesized from the mRNA by the enzymes: reverse transcriptase and DNA polymerase, to form complementary DNA (cDNA). Next, the cDNA is mixed with the qPCR master mix which is given with the kit and then the mixture was spread equally on the PCR arrays. Lastly, real-time amplification data was collected with the help of instruments software and the fold changes in expression were analyzed (SAS Biosciences).

In our experiment we are going to use PCR Array and treat OVCAR3 a dsRNA sensitive cell with various therapeutic agents (Van et al., 2012). There will be two types of cells: untreated and treated. The untreated cell will serve as a base line for the treated cell. The three treated cells will be stimulated with dsRNA, paclitaxel and both. The experiment will be done at least three times to get precise results. As shown in Figure 1, OVCAR 3 cells will be treated for 48 hours with dsRNA, paclitaxel and both with ED50 concentration of 4X since it has the highest cell death statistically (Van et al., 2012). Once the cells are cultured with the different types of drugs, a PCR Array will be performed to measure mRNA expression levels (SAS Biosciences).

Once the experiment is performed, the results will be analyzed. The combined treatments should have more of a response then the individual treatments and cause synergistic cell death of ovarian cancer cells. To determine which treatment is more effective, specific pathways or proteins activated by both the combined and individual treatments will be identified. By identifying specific pathways, patients retaining these functional pathways will benefit from the treatment. Also the downregulation can be compared of the individual, combined and untreated treatments. By comparing the decrease in the cellular components, the effectiveness of each treatment can be found. Potential pitfalls with this experiment are that some patients will not respond to the drugs used or they might have to give less doses of drugs if they have too much of a response. Alternative approaches can be blocking the apoptotic pathways of paclitaxel to examine the impact on the dual synergistic treatment on apoptosis of ovarian cancer cells. Furthermore different drugs can be used with dsRNA to increase apoptosis.

Figure 2: PCR Array Procedure

Discussion:

Many treatments like cytoreduction surgery followed by chemotherapy have been used to treat patients but none of them ensured the elimination of tumor. After each surgery, chemotherapy, with certain drugs, was assigned according to the patient and the cancer, but eventually the tumor would grow back. If too much of a dose was given then it would harm the patient by killing its regular cells with the tumor cells. In that case, different drugs like cisplatin, bevacizurnab, taxane, and docetaxel are used in chemotherapy or a different approach which was mentioned earlier in the paper, intraperitoneal chemotherapy delivery can be used. In order to give smaller amounts of doses and cause more cell death the combination of dsRNA and paclitaxel were used. Not only can the patient be treated with fewer doses but it can also have a higher cell death ratio resulted by the synergistic effect of two therapeutic agents treated together. In order to assure the presence of the synergistic effect, specific pathways of dsRNA and paclitaxel that lead to apoptosis were found. For each patient these essential pathways will serve as biomarkers. Also, they will open pathways to many other treatments and ensure apoptosis of ovarian cancer cells.

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