Types Of Epithelial Ovarian Cancer

Ovarian Cancer is one of the most dangerous cancers found in woman. Epithelial Ovarian Cancer (EOC) is the malignancy within the epithelial cells in the ovary. This consists of two types, type I and type II, in this review the differences between the two are discussed and highlighted in terms of treatment and diagnosis. Early detection of EOC is incredibly difficult due to the non-specificity and selectivity of current biomarkers approved by the FDA such as CA125. This review highlights future biomarkers that could be used individually or as a panel to detect EOC in the early stages, thus increasing the survival rate. It has been suggested that type I and type II are so different that personalised treatments are needed for each patient. Problems such as chemoresistance can arise in many patients which is why individual treatment is suited. Proteomics and Genomics are highly beneficial in identifying new biomarkers. By investigating gene expression and protein profiles there will be a profound impact on patients with EOC in relation to the detection and treatment. Genetics has played a huge role in discovering biomarkers for disease however current research suggest that genomics can be limiting compared to using proteomics. Proteins contain many post translational modifications which cause huge variability. Comparing proteins in diseased patients with healthy patients can result in potential biomarker discovery. These future developments will shape clinical diagnosis and treatment in the future years to come, especially woman with EOC.

Introduction

Cancer is disease which describes the uncontrollable cell growth of certain cells in the body and their invasion into different tissues. Most causes of malignancies occur by alterations or mutations to the DNA. (pecorino, 2016) Each patient with cancer is unique, two patients with the same type of cancer may receive two different treatments that are specific to them. Ovarian cancer is the cancer of the ovaries in woman, however, there are different types of cells within the ovaries that can be affected. The epithelial cells that cover the ovaries is the most common source of malignancies. ‘Epithelial ovarian cancer (EOC) occurs in 65-90% of all cases, usually seen in older woman.’ (A & A., 2010) Type I epithelial tumours have a higher survival rate than type II epithelial tumours. Type II epithelial tumours account for 75% of all epithelial ovarian cancers. ‘EOC causes the highest number of deaths in woman due to a gynaecologic malignancy and has a very low survival rate of 44%.’ This low survival rate may be due to the high occurrence of chemoresistance seen in EOC patients. (Xhang & Zhang, 2016) Ovarian cancer is mostly asymptomatic in many woman, so early detection is difficult. However, when symptoms are witnessed they usually consist of persistent abdominal pain, bloating and decreased appetite. (Matz M, et al., 2017) There are different stages when being diagnosed with EOC. In stage I the tumour is contained within one or both of the ovaries. In stage II the tumour has spread to the pelvic region. Stage III describes the growth outside of the pelvic region into the abdominal cavity or lymph. In stage IV the tumour is located in the liver. (Page, et al., 2010)

In recent years there has been a development in proteomics and biomarker potential to increase survival rate. Proteomics is the study of all the proteins in an organism and can be applied to medicine through identification of proteins as markers of diseases and targets of new drugs. This aids the development of specific treatment for individual patients to increase

effectiveness. (Mishra, 2011) The measurement of biomarkers shows if the level of a certain protein falls within a normal or abnormal range. They can be detected by a variety of techniques such as mass spectrometry, protein microarray and 2D electrophoresis. Currently only a few proteins are approved by the FDA as biomarkers of different human diseases.

(pecorino, 2016) Cancer is also a disease of gene dysregulation and studying genes in diseased patients can provide potential targets for therapeutic intervention. (Kurman & Shih, 2010)

At present, current testing and screening procedures would include the CA125 tumour antigen measurement, the transvaginal ultrasonography (TVU) and the pelvic examination. However, these have shown to be unsuccessful in early detection of EOC and are often associated with false positives. It has been demonstrated by the Prostate, Lung, Colorectal and Ovarian Trial that yearly screenings using these techniques has not shown any increase in survival rate but increases unnecessary surgical procedures. (Xhang & Zhang, 2016) This indicates a need for new developments in early detection methods for EOC.

Due to lack of specificity of current biomarkers for ovarian cancer early detection in woman is difficult. Lack of symptoms and poor screening for this disease can lead to the majority of cases having late stage IV ovarian cancer, which shows a low survival rate in most woman. (Kuk, et al., 2008) Epithelial ovarian cancer is the most dangerous cancer found in woman with more than 204,000 cases every year around the world, this accounts for 4% of all cancer cases. (Zhang, et al., 2010) It is also the leading cause of gynaecologic malignant death. Despite it being more common than breast cancer it is three times as lethal. In the United States alone 16,210 woman will die from the disease. As a lot of these cases are detected when the disease is advanced, less than 10-15% will remain in permanent remission. This is all due to the difficulties with early detection and diagnosis, showing how important proteomics and biomarkers are in the prognosis of the disease. (Gil, et al., 2005)

There are many different techniques to test and analyse the proteomic profile. Normally proteins are studied from a blood sample or urine sample. However, the protein profiles in urine is less complex and more stable than in blood. Urinary tests are also less invasive than taking a blood sample. (Vanderhyden & C., 2006) Different techniques used to analyse the protein would be 2D gel electrophoresis. This separates the protein according to characteristics such as size and charge. Mass Spectrometry is also used to calculate the mass and charge of specific proteins. (Zhang, et al., 2010) Samples are usually done from a control and an ovarian cancer patient to compare protein differences. Comparing changes that occur in ovarian cancer patients but not in healthy patients can lead to identifying different urinary biomarkers elevated in ovarian cancer. (Vanderhyden & C., 2006) Bioinformatics can then be used as a computational method to analyse the proteins. This can covert proteomics data into knowledge which can then be applied to the development of methodology. The copious amount of information provided is then complementary to genomic research. The combination of both will impact developments of diagnostics and therapeutics for EOC in the future. (C.S.Cho, 2007)

CA125 is the most commonly used biomarker in EOC patients, it is used in screening for early detection of the disease and monitoring of the disease. ‘CA125 is a high molecular weight mucinous glycoprotein (MUC16) identified in 1981.’Nearly 80% of ovarian cancer patients express this in their blood serum. (Moore, et al., 2010) A CA125 level less than 35U/ml is considered to be a normal amount, however there can be many false positives generated. (A &

A., 2010) A decrease CA125 during chemotherapy can indicate the success of treatment for the patient. However this screening test for EOC is not sufficient as it lacks the high sensitivity and specificity needed. (Cho, 2007)

When testing for biomarkers in a patient it is advised to use a selection of biomarkers. When individual proteins are identified as biomarkers they are not as good at predicting disease than using a set of proteins that all act as biomarkers. For example; ‘an approach taken by Han and Colleagues, where 165 combinations of MUCIN16’ were examined to see if they could show differences between benign conditions or malignant conditions. This was used to find the optimal combinations of different biomarkers used together. (Enroth, et al., 2019) For example, a combination of four proteins are often used as indicators for ovarian cancer, proteins such as leptin, prolactin, osteopontin and insulin like growth factor II. These proteins by themselves do not indicate ovarian cancer. (Mishra, 2011)

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There are genetic variations between type I and type II tumours. Type I are less aggressive and are more genetically stable than type II tumours. Type I tumours contain KRAS, BRAF and ERBB2 mutations whereas TP53 mutations are infrequent. The opposite is seen in type II tumours, they are highly genetically unstable and show a high frequency of TP53 mutations. It has been suggested that the different types of EOC develop through different molecular pathways. (Kurman & Shih, 2010) It is thought that EOC type II doesn’t develop from the ovary itself but from fallopian tube precursors and it is not confined to the ovary. (Ueland, 2017) It has been suggested that Type I ovarian carcinomas originates from a benign precursor lesion such as endometriosis. Type I tumours are generally slow growing and attain a large size while still confined to the ovary. (Koshiyama, et al., 2014) Numerous studies have argued that the development of a biomarker screening test for type I is not urgent. However, it is type II tumours that need to be targeted for screening using biomarkers. The goal for type II tumours in screening is ‘detection of low volume not low stage disease’. This can be achieved by the findings of ‘a panel of sensitive and specific biomarkers that are expressed early in ovarian carcinogenesis.’ (Kurman & Shih, 2010) There are many advantages and disadvantages to both proteomics and genomics when it comes to identifying and treating diseases such as ovarian cancer. Proteomics is relatively new and still very much in development compared to genomics. A downfall when dealing with proteomics is using complex samples and body fluids. Proteins also have different isoforms and can undergo post-translational changes, such as glycosylation, these can then increase their complexity. However proteomic profiling increases the chances of early detection of a disease such as EOC. (Kavallaris & Marshall, 2005) New techniques for specific and selective protein analysis in tissue and fluids will have an immense impact on clinical diagnostics in the future. Profiling different tissues is necessary to discover any protein or molecular change that could indicate the mechanism of disease. (Demkow, 2010) It has been discussed in many papers that the emphasis in diagnostics is shifting from genomics to proteomics in recent years. DNA and RNA have shown to be easier to work with however there are limitations to the information gotten from DNA and RNA. Genomics does not provide an accurate profile of proteins structure and function or the abundance of a protein. It is thought that both genomics and proteomics together can develop the necessary new disease biomarkers to prevent, treat and diagnose diseases such as ovarian cancer. (C.S.Cho, 2007)

It has been discovered that genetics is needed when uncovering what occurs in EOC patients.

Treatments such as chemotherapy have shown to initially show a clinical response however in type I tumours there is high chemosensitivity. Currently, 60-80% of patients with EOC respond best to a combination of platinum and taxane based chemotherapy leading to remission. However within 6 months patients can develop an acquired platinum resistance causing treatment failure. (Xhang & Zhang, 2016) Treatment of type I tumours with chemotherapy shows a greater struggle compared to type II due to its slow growing nature. New advances are needed for type I treatment.

Genes that cause deregulation of signalling pathways could indicate potential targets to aid therapeutic treatment development. (Kurman & Shih, 2010) The interaction between the drug and the tumour may lead to up or down-regulation of genes in these pathways that could cause variation in response to chemotherapy. A study was done by Queens University and the Ottawa Hospital Research Institute (OHRI) on 28 high grade EOC tumours. Two over-expressed (IGF1 And ZFP36) and two under-expressed genes (MCM8 and ZNF83) were found and analysed on PCR. Signalling pathways were studied such as E2F and estrogen receptor. The associated functions with these were ‘cellular development, cellular growth and proliferation.’ It was noted that the presence and intensity of IGF1 in pathways indicates its potential role in chemoresistance. Studies have suggested that the expression of the IGF1 receptor can lead to the mechanisms of cancer and chemoresistance in cancer cells. The increased expression of IGF1 indicates a need for gene expression predictive biomarkers in the pathway. (Koti, et al., 2013) There are only two biomarkers approved by the FDA for EOC diagnosis. This suggests an imperative need for updated diagnostic techniques.

Exosomes have shown to be a potential source for biomarkers and play an important role of cell to cell communication. Exosomes are secreted by various cell types, they are spherical bilayered proteolipids and are abundant in various proteins. It may be the case that exosomes can transport some tumour associated proteins which originate from cancer cells. A study suggested that two genes FasL and TRAIL were located in exosomes and contributed to apoptosis of immune cells. Fudan University in Shanghai extracted exosomes from EOC patients and analysed the various proteins. The proteins were analysed and purified from exosome samples by mass spectrometry. It was discovered that 4 genes were selected as potential biomarkers as they all contributed to coagulation and apoptosis. FGA was one of these biomarkers, its levels were significantly elevated and produced the highest area under the curve (AUC). This biomarker shows promising potential as a future FDA approved biomarker. (Wei, et al., 2018) There is also evidence suggesting that exosomes have a role in communication between cancer cells and immune cells and the suppressing of the activation of T cells, Natural Killer cells and monocytes. (Nakamura, et al., 2019)

Exosomes have a role in the process of peritoneal dissemination. This describes the form of malignant progression. (Ricardo, et al., 2018) Cancer cells spread from the primary site, the ovary and enter the peritoneal cavity. ‘Exosomes secreted by the ovarian cancer cell promote each step of peritoneal dissemination.’ (Nakamura, et al., 2019) This is summarized in figure 4 below:

A marker under investigation that shows contrasting observations is Ki67. Ki67 is a proliferation marker, a nuclear protein in cells present throughout G1, S, G2, and M in the cell cycle. One study suggests that it has been over expressed mostly in cancer tissues than in benign tissues. High expression of this protein has shown poor survival in many patients. However other studies have observed that other variables may influence the presence of Ki67 in cancer cells and may

not correlate with the degree of the malignancy. (Page, et al., 2010)

Another potential EOC biomarker are MicroRNAs. These are non-coding regions of RNAs and conduct regulation of gene expression through mRNA degradation. This can then have an impact on certain processes in the cell such as proliferation or apoptosis. A vast amount of research has indicated that there is high levels of expression of miRNAs in EOC and may act as tumour suppressor genes. Further study into how miRNAs regulate expression will provide the development of future biomarkers for EOC.

Processes such as microarrays have been able to analyse miRNAs, a study concluded that in EOC samples miR200a, miR200b and iR200c were overexpressed compared to the control. They are attractive as biomarkers compared to mRNAs due to the fact that they are contained within a protective layer of serum and plasma which allows expression patterns to be taken directly from the serum. (Zhang, et al., 2011)

Recent studies have suggested that Lysophosphatidic acid (LPA) could have potential as a biomarker, however, it is evident that there are conflicting results. In the majority of studies LPA plasma levels were elevated in patients with EOC. LPA may contribute to the proliferation and cell survival of EOC. It has subspecies including lysophospholipid (LPL) and Lysophohphatidylinositol (LPI). All three together were investigated as a potential panel of biomarkers. LPA levels (including its subspecies) achieved 91.1% in sensitivity and 92.6% in specificity. The study confirmed that in early stage disease of EOC elevated levels of LPA are seen. Continuous studies are being done to identify effectiveness as a biomarker when combined with other markers such as CA125. (Sutphen, et al., 2004)

A potential source of biomarkers could also be obtained by looking at DNA methylation. This is often seen in tumours of EOC and is identified as the earliest molecular change in malignancies. Methylation in genomes holds promise as potential biomarkers for the study of EOC progression and response to therapy. A gene which undergoes promoter methylation is BRCA1, it is associated with loss of its expression. Malignancies can be correlated with high levels of BRCA1 methylation. This gene has suggested a possible role in platinum based chemotherapy resistance. Studies have suggested that BRCA1 may serve as a biomarker for aggressive EOC. A similar situation is seen with HOXA9 which is hypermethylated in the promoter region. ‘HOXA9 was methylated in 51% of EOC tumours with significantly higher methylation frequencies identified in early stage tumours.’ This methylated gene could potentially serve as a biomarker as its methylation correlates with the increasing risk of EOC in previous studies. It is clear that DNA methylation should be looked at in a panel of biomarkers rather than individual methylation to achieve high specificity. (Gloss & Samimi, 2014)

Conclusion

To conclude Epithelial ovarian cancer is an underdeveloped disease in relation to its early diagnosis and treatment. With the aid of new technologies integrating proteomics and genomics, gene expression and protein profiling will develop more potential biomarkers.

Extraction of DNA and proteins from the cell is done followed by high throughput processes such as mass spectrometry and microarrays. Potential biomarkers have been identified and continue to be studied in experiments. These include exosomes, miRNAs and methylated genes

such as BRCA1. Biomarkers can be genomic or proteomic. However, the majority of studies have indicated that proteomics will be extremely important in the future for developing biomarkers. Both genomics and proteomics together are complementary and the clinical diagnosis of diseases such as EOC will be changed when new technologies are produced.

Due to the complexity of EOC it will be unlikely that only one biomarker will be able to detect EOC with high specificity and sensitivity. However, there is hope that with the two current FDA approved biomarkers, newly developed biomarkers may create a panel of ideal markers for EOC patients. With these advances, increased specificity and sensitivity of biomarkers will then be able to diagnose and treat early onset EOC in patients.