

Molecular Prognosis of Epithelial Ovarian Cancer

Observations from Current Literature

Cancer of the ovary is both the most prevalent and lethal form of gynaecological carcinoma. More than three quarters of women afflicted have disseminated disease at the time of diagnosis and receive treatment which is usually a combination of debulking surgery and chemotherapy. The most effective chemotherapy agent against ovarian cancer (OvCa) is cisplatin – also used for lung, head and neck, bladder and testicular cancers. Response rates to this drug vary from 40%-80% and it is often used in conjunction with other treatments (eg. Paclitaxel) to achieve a subtle increase in the proportion of patients successfully treated.

This disease continues to be a focus of intense research around the world because of the significant fraction of women whose initially responsive tumors develop resistance to all available chemotherapy regimens. Drug-resistant disease is eventually observed in more than

75% of cases after four years from diagnosis and consequently the 5 year survival rate in Australia is around 42% - lower than the mean combined survival rate for all female cancer patients o 63%(Australian Institute of Health and Welfare. and Australasian Association of Cancer Registries. 2001). While survival times have significantly increased over the past 20 years this has unfortunately not correlated with an equally significant improvement in the cure rate(Engel, Eckel et al. 2002), thus the genetic changes accumulated by chemotherapy-resistant ovarian cancer cells remain of keen interest.

Alterations in the oncogene TP53 and its downstream targets p21 (cell cycle inhibitor), BAX (apoptosis agonist) and BCL-2 (apoptosis antagonist) are often observed in OvCa, however there is still debate concerning the prognostic function of these changes. Schuyer et al used a range of molecular and immunohistological methods to examine the relationship of these genes and important clinico-pathological variables, including outcome and response to platinum-based chemotherapy drugs including cisplatin(Schuyer, van der Burg et al. 2001).

Interestingly, while P53 mutations are present in up to 50% of epithelial ovarian cancers, there was no observed correlation with increased rate of progression or death, nor with expression of p21 or BCL-2 in this study. Higher TP53 protein expression levels could, however, be correlated with shorter overall survival rate (P=0.03). Factoring TP53 mutation and over-expression resulted in a more significant correlation with overall survival than the expression data alone (P=0.08), as observed in other studies (Wen, Reles et al. 1999). The TP53 target gene, BAX, was significantly linked to progression-free and overall survival (see Figure 1). Furthermore, patients with expression of both BAX and BCL-2 exhibited longer survival times than those whose tumors express BAX alone. The authors conclude that high expression of BAX may therefore be a potential independent prognostic indicator for this disease.

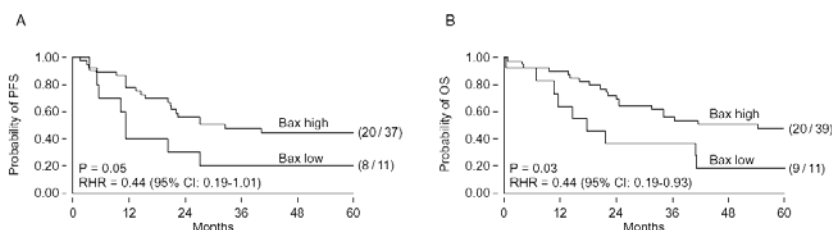


Figure 1: (a) Progression-free and (b) overall survival rates for OvCa patients classified by BAX expression. Taken from(Schuyer, van der Burg et al. 2001).

Expression of P21/WAF1, a tumor suppressor gene whose expression is inversely correlated with TP53, has been associated with high tumor grades and late FIGO stages(Anttila, Kosma et al. 1999). DNA damaging agents that result in cell cycle arrest in the G1 phase of wild-type P53 cells are capable of inducing the p21/WAF1 gene. Antilla et al used immunohistochemical profiling of over 300 ovarian tumor specimens to explore the relationship between expression of this p21/WAF1 and patient outcome. Statistical analysis of expression levels and patient clinical information revealed that high level expression of p21/WAF1 resulted in lowered levels of cellular proliferation. In a univariate approach, the gene appeared to be a negative prognostic factor. Patients whose tumors had minimal or no expression appeared to have a higher risk of tumor recurrence after treatment and shorter disease-free and overall survival rates, particularly for those positive for p53 also. Whilst not statistically significant, there was a trend towards higher p21/WAF1 expression in patients who had a complete response to chemotherapy.

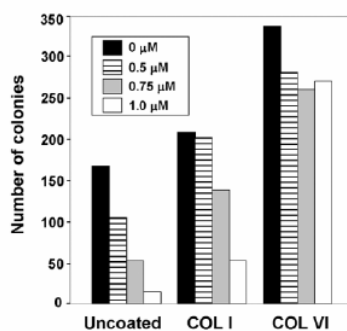


Figure 2: Number of survival colonies of OvCa cell line A2780 plated on collagen substrates after cisplatin exposure. Taken from (Pitchford and Page 2003).

Unfortunately none of the variables studied by Schuyer et al(Schuyer, van der Burg et al. 2001) could be associated with tumour response to platinum-based chemotherapy, suggesting that chemosensitivity may be controlled by other genetic changes. Variation in chemotherapy regimens between clinicians and hospitals, as well as the complexity of quantifying response, add to the difficulty of relating molecular changes to variations in drug sensitivity. Serial analysis of gene expression (SAGE) profiling is one method that has been employed to pursue this multi-factorial trait of OvCa. Many of the genes that exhibit differences in expression levels between cisplatin-resistant and sensitive cells are related to cell interactions with their microenvironment(Pitchford and Page 2003). The hypothesis that resistance to chemotherapy may be brought about by direct modifications of the extracellular matrix (ECM) was supported by experiments in which cisplatin-sensitive cells cultured in the presence of collagen VI. This protein

is the product of one of the most differentially expressed genes observed by the SAGE (COL6A3) (Pitchford and Page 2003). Cells grown in the presence of collagen VI developed a 15-fold increase in chemo-resistance (see Figure 2), indicating that ECM remodelling may be a key step in the process of a tumor developing resistance to previously effective treatments. Finally this study showed with the use of tissue arrays (120 cases in total) that expression of COL6A3 in primary ovarian tumors is also correlated with tumor grade. This pathological measure has been shown to relate to chemotherapy response and overall survival rates.

The gene KLK4 (Obiezu, Scorilas et al. 2001) (Higher Human kallikrein gene 4) has also been associated with disease progression and survival time in OvCa. KLK4 has been implicated in other hormonally regulated cancers, including those of the breast and prostate. In 147 OvCa samples, expression of the gene was found 55% of tumours and there was significant association with tumor grade and stage. Overall the authors of this study concluded that KLK4 expression was higher in tumors with a more aggressive phenotype, generally translating to an increased risk of relapse and death. When tested against chemotherapy response rates, a correlation between positive expression and lack of treatment efficacy was detected. Interestingly, it was noted that positive KLK4 expression in Grade 1 and 2 cases indicated a 2.5-fold increase in relative risk of relapse, yet the same degree of up regulation was not significantly predictive of relapse in grade 3 tumors (see Figure 3).

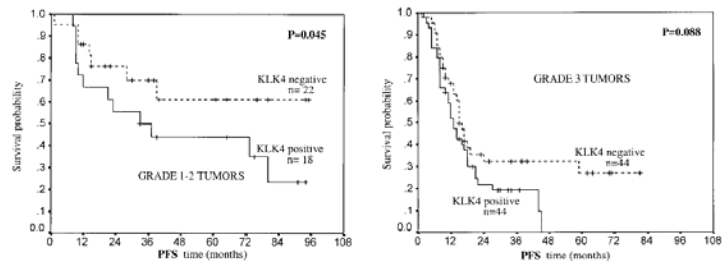


Figure 3: Variation in rate of tumor relapse between grade 1-2 and grade 3 tumors by KLK4 expression. Taken from (Obiezu, Scorilas et al. 2001)

The Fanconi anemia-BRCA pathway has been implicated in the molecular changes occurring in cisplatin-resistant OvCa. Interruption of this genetic pathway ultimately appears to lead to the development and selection of drug-resistant cancer cells (Taniguchi, Tischkowitz et al. 2003). This pathway is comprised of six genes associated with Fanconi anemia syndrome (FANCA, -C, -D2, -E, -F & -G) plus BRCA1 and BRCA2 and regulates cellular reaction to cisplatin and other DNA cross-linking substances. Cisplatin resistance in OvCa cell lines can be attributed to initial methylation-induced inactivation and subsequent demethylation of FANCF (Taniguchi, Tischkowitz et al. 2003). As this work was carried out using OvCa cell lines it may require validation using other methods such as expression profiling of RNA extracted from human tissue. Indeed, other studies have shown considerable molecular differences between cell lines and ovarian tumors on the basis of hierarchical clustering and multi-dimensional scaling (MDS) with data generated from cDNA microarrays (Sawiris, Sherman-Baust et al. 2002) as shown in Figure 4.

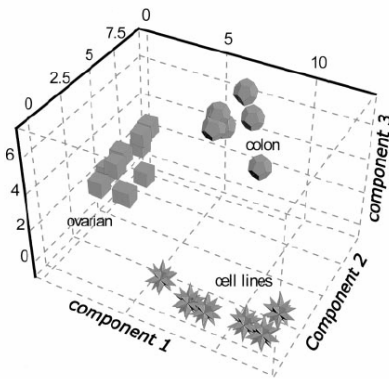


Figure 4: MDS 3D plot of cell line (stars), OvCa (cubes) and colon (octagons) tumor samples. Taken from (Sawiris, Sherman-Baust et al. 2002)

Using RT-PCR profiling of 120 epithelial cancers the authors describe a highly significant relationship between this gene and prolonged survival. Patients who lived for more than 5 years had 2.2-fold higher expression of this gene than those who died within 12 months of diagnosis. This gene is a member of the "death ligands" and a member of the apoptotic pathway. Another study has demonstrated the combination of TRAIL and chemotherapy lead to a significant increase in apoptosis and growth inhibition of OvCa cell lines and propose the clinical use of this treatment combination (Cuello, Ettenberg et al. 2001).

It is clear that the difficulty of successful early detection and high rate of treatment resistance remain two of the key challenges in OvCa treatment and research. If these hurdles can be even partially overcome through an increased understanding and manipulation of the underlying molecular changes many lives may be saved from this aggressive and widespread disease.

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