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Review

The role of gene expression profiling in the clinical management of ovarian cancer

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ABSTRACT

Several studies have addressed the clinical value of gene expression profiling in the field of ovarian cancer. This paper reviews the current status of knowledge that can be derived from such studies. Gene expression profiles can be used to reveal sets of genes that can distinguish normal ovarian tissue from invasive ovarian carcinomas. Independent validation of these sets may result in the identification of (a set of) markers valuable for the detection in an early stage.

Microarray analysis has shown that different histological subtypes of ovarian cancer might be partly reflected by a different aetiology through the deregulation and activation of different pathways. In addition, this heterogeneity could therefore also lead to different tumour behaviours.

Worldwide, the combination of paclitaxel and platinum chemotherapy has been incorporated in the standard protocol for the management of patients with advanced stage ovarian cancer, although the outcome in individual patients is uncertain. Gene expression profiling was found to be a prognostic tool with respect to chemosensitivity and had a predictive performance of 78–86%. With increasing numbers of data from published reports, access to these data for the reproducibility of its results and pooling becomes more and more important and will possibly lead to more individualisation of therapy.

1. Introduction

Epithelial cancer of the ovary is the sixth most common cancer in women, affecting approximately 1 in 70 women in the developed world, and is the leading cause of death from a gynaecological malignancy.[1] The age at diagnosis, extent of disease (as expressed by FIGO stage), success of primary surgery, and the histopathological features of the tumour are important prognostic markers.[1–5] Several single genes have been suggested as markers for prognosis, but are still under investigation (Table 1).[6–13] The present 5-year survival rates are about 80–90% for stages Ia–Ic, 70–80% for stages IIa–IIc, 30–50% for stages IIIa–IIIc and 13% for stage IV. However, the majority (>60%) of ovarian cancers are found in advanced stage (FIGO stages III/IV).[3]

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Table 1 – Ovarian cancer markers associated with prognosis

Marker	Incorporation in the clinic	Determinants	References
Age at diagnosis	Established	Patients older than 69 years of age exhibit significantly poorer survival than those younger, even after correction for stage, residual disease, and performance status	[4]
Histological subtype	Established	Clear cell carcinomas are more likely to be chemoresistant and associated with a poor prognosis; advanced stage mucinous carcinomas have a bad prognosis	[1]
Surgical outcome	Established	Patients with residual tumour 1 cm or less in diameter have higher survival rates	[2]
FIGO stage	Established	Patients with a stages I–II have a 5-year survival rate of 70–90%	[3,5]
BRCA status	Under investigation	Both a worse and a better survival are noted for BRCA ovarian cancer patients	[7,10]
Epidermal growth factor receptors	Under investigation	The prognostic impact of EGFR overexpression is controversial as a predictor of survival	[8,10,11,13]
P53	Under investigation	A correlation was shown in an univariate analysis between P53 mutation and chemosensitivity and a shortened survival	[9,12]
BCL-2	Under investigation	BCL2 has been associated with an improved prognosis	[6,9]

Except for those with subgroups within stage I tumours, patients are treated with chemotherapy after surgical exploration and tumour debulking. However, no good predictor of who will benefit from chemotherapy is available. Only 50% of the patients with an initial response to chemotherapy will still be alive after 5 years. Drug resistance may (partially) explain this effect, which is thought to be caused by pharmacokinetic, tumour microenvironmental or cancer-cell specific factors.[14] Single molecular markers, e.g. expression of genes that are known to be involved in drug resistance in experimental systems, have not kept their promise in the clinical setting.[15,16]

Thus, the prognostic and predictive parameters as described are far from precise, nor are the current chemotherapy regimens highly effective, which emphasises the need to identify new markers including those that could potentially be used to develop new targeted therapies. It is believed that the gene expression patterns of tumours could fulfil this demand as tumour behaviour is determined by the integrated action of many genes. Expression of thousands of genes can nowadays be studied simultaneously by the use of different kinds of microarrays (Table 2).

Recently, several research groups have taken this approach and established gene expression profiles of ovarian tumours and normal ovarian tissue, different subtypes of ovarian cancer or of those that are responsive to therapy. This minireview will summarise the current profiles that address prognosis and therapy response prediction.

2. Methods

A systematic literature study was performed to identify articles published until December 2005. A Pub Med search was performed using MeSH terms 'ovarian neoplasm', 'microarray' and 'gene expression profiling'. We selected clinical studies in which cDNA or oligonucleotide microarray analyses were described that distinguish normal ovarian tissue from ovarian cancers, that classify cancers into histopathological or genetic subtypes or that predict prognosis or tumour response to treatment. Significance of each of the papers is discussed. A statistical meta-analysis of all reviewed articles was not possible due to the lack of randomised studies, small groups and the diversity of the studies.

Table 2 – Common different types of microarrays and hybridisations used

Platform	Features	Hybridisation
cDNA	cDNA clones of 500–5000 base pairs are spotted at defined positions on a glass slide.	RNA from a test and reference sample, isolated from normal or tumour tissue, is converted into cDNA or cRNA and labelled with a fluorescent dye (red or blue dye). After hybridisation the relative expression levels are measured. Expression levels of the test samples are assessed by determining fluorescent intensities and compared to expression levels of a reference sample that is labelled with the complementary dye
Oligo array	Oligonucleotides of 20–80 base pairs are synthesised either in situ (on-chip/glass slide) or by conventional synthesis followed by spotting on a glass slide	Long oligonucleotide platforms use the above described method. Hybridisation of one labelled sample (cDNA or cRNA) is used in short oligonucleotide platforms

3. Usage of gene expression profiling in the aetiology of ovarian cancer

At this moment little is known on the molecular changes that are associated with the development from normal ovarian tissue to invasive ovarian cancer. No lesion is clinically recognised that immediately precedes ovarian cancer. The late onset of symptoms (in patients with invasive cancers) makes early detection difficult. Surface epithelial tumours account for 50–55% of all ovarian tumours, and their malignant forms account for approximately 90% of all ovarian cancers in the western world.[17] Four major histological epithelial subtypes are known: serous, mucinous, endometrioid, and clear cell. The serous and mucinous ovarian carcinomas primarily originate from the mesothelial lining of the ovarian cortex or cortical inclusion cysts. The metaplastic change of the surface mesothelium into a Müllerian phenotype may occur either before or during the genesis of malignancy. The aetiology of endometrioid and clear cell ovarian cancers is strongly associated with endometriotic deposits.[18] Hogg *et al.*[19] discuss in the context of efficacy of screening, the evidence that stage I ovarian carcinomas are biologically different from advanced stage carcinomas. Following the line of argumentation for the dualistic models of serous carcinomas described by Singer *et al.*,[20] Hogg *et al.* propose two separate models for the development and progression of epithelial ovarian cancer in general. The first model includes endometrioid, clear cell mucinous and well-differentiated micropapillary serous cancers. Most (at least 75%) of these are either stage I or stage II at the time of clinical presentation. They have a slower onset and are detectable by screening. The second model includes high-grade serous cancers and the great majority is advanced at the time of diagnosis. This type has a multifocal origin, progresses rapidly and screening with the current modalities is of little value.[19] As yet, the practical value of this modelling is limited due to the great variety of clinicopathological manifestations of ovarian cancers.[17] Thus, these models only partly apply to tumour behaviour and are at present not sufficient enough to use in clinical practice.

Therefore, can microarray analysis be of help in revealing pathways that lead to ovarian cancer? Several studies demonstrated that gene expression profiles can be used to derive sets of genes that can distinguish normal ovarian tissue from invasive ovarian carcinomas.[21–27] Most studies listed in Table 3 fo-

Table 3 – Microarray studies and the comparison between normal ovarian tissue and malignant ovarian tissue

Study	N	Ovarian tissue	Microarray type	Technical validation	Performance in an independent validation	Details
Schummer *et al.*[25]	16	10 OSPC *versus* 6 NOVA	Nylon membrane cDNA	8/43 known genes with RT-PCR	–	HE4 showed a tumour specific expression pattern.
Welsh *et al.*[27]	31	27 OSPC *versus* 4 NOVA	Glass wafer oligonucleotide	1. CD24, HE4 and LU with RT-PCR 2. UNIgene database	–	Distinction between normal and cancer tissue
Tonin *et al.*[26]	6	4 OSPC cell lines + 1 OSPC *versus* 1 NOVA cell line	Affymetrix oligonucleotide	Northern blot analysis	–	The expression pattern of the cell line with indolent disease resembles NOV-31 while profiles of samples derived from patients with more aggressive disease showed different expression profiles
Hibbs *et al.*[22]	87	20 OSPC, 17 meta's OSPC and 50 NOVA	Affymetrix oligonucleotide	7/66 genes with IHC in 45 ovarian tissues	–	Beta8 integrin subunit, claudin-4 and S100A1 provided the best distinction between normal and cancer tissue
Santin *et al.*[24]	17	10 OSPC + 2 OSCP cell lines *versus* 5 NOVA	Affymetrix oligonucleotide	1. Four genes with qRT-PCR 2. Flow cytometry 3. TROP-1 and CD24 with IHC	–	Overexpression of TROP-1/Ep-cam and CPE epithelial receptors in ovarian cancer
Donninger *et al.*[21]	43	37 OSPC *versus* 6 NOVA	Affymetrix oligonucleotide	1. 12/1191 genes previously known 2. 14 genes with qRT-PCR	–	1191 genes were differentially regulated between ovarian cancer and normal tissue specimen
Lu *et al.*[23]	42	42 OSPC *versus* five pools of NOVA	Affymetrix oligonucleotide	1. RT-PCR 2. Five genes used for IHC	–	Four genes (E2F3, HN1, NOTCH3 and RACGAP1) separate normal from cancer tissue. Using RDPA, the combination of CLDN3 and VEGF also distinguishes all cancers from normal tissue

Abbreviations. OSPC, ovarian serous papillary carcinoma; NOVA, normal ovarian epithelium; (q)RT-PCR, (quantitative) real time-polymerase chain reaction; IHC: immunohistochemistry.

cused on gene lists that are differentially expressed between ovarian cancer samples and normal ovarian tissue, rather then attempting to classify samples into these two groups using specified gene expression profiles.[21,22,24–27]

Although the cohorts studied were small, the best available results were the more recent reports of Santin *et al.*[24] and Lu *et al.*[23] The first report concerns 12 malignant ovarian serous papillary carcinoma cell lines (OSPC; 10 primary and 2 established) and 5 normal ovarian epithelium (NOVA) cell lines. In the hierarchical clustering by using 299 genes, which can be of help in discriminating different groups, it was shown that all 10 primary OSPC clustered tightly together. Expression of some of the 299 significantly differentially expressed genes was validated by quantitative RT-PCR, flow cytometry and immunohistochemical staining. CD24, TROP-1/EPCAM and Claudins 3 and 4 were among the most overexpressed genes in OSPC as compared to NOVA. It was suggested by the authors that these genes may be used for the development of novel therapeutics.[24] CD24 protein expression was also described by others, as determined immunohistochemically in 9 normal ovaries and 69 epithelial ovarian tumours of different types. In invasive ovarian carcinomas, they found membranous expression in 84% of the samples. Moreover, CD24 was found to be independently associated with shortened patient survival in a multivariate analysis.[28] Lu *et al.*[23] explored the use of recursive descent partition analysis (RDPA) in which a sample is assigned to a diagnostic category of cancer or normal tissue based on a gene expression decision tree. 86 genes were identified that were 3-fold upregulated in ovarian cancer compared to normal ovarian cells. Of these, the combination of four genes: E2F3, HN1, NOTCH3 and RACGAP1, or the combination of CLDN3 and VEGF could perfectly separate tumour from normal cells.[23]

Taken together, numerous genes have been identified that were shown to be up- or downregulated in the process of carcinogenesis. A molecular distinction between normal tissue and malignancy was found, but for a firmer confirmation of the models proposed by Hogg *et al.*,[19] profiling of histological subtypes preferably at different stages of disease is required. Can indeed different histological epithelial subtypes be distinguished by microarray profiling, substantiating different aetiologies? It is known that the serous subtype represents approximately half of all malignant epithelial tumours, the endometrioid subtype about 17% and the clear cell and mucinous carcinomas account for 8% and 13%, respectively, of all ovarian carcinomas.[3] In pathology, an endometrioid adenocarcinoma may be difficult to distinguish from a serous carcinoma, especially when the tumour is poorly differentiated. The stage distribution at diagnosis of endometrioid carcinomas (50% are stages I/II) differs from that of serous carcinomas (27% are stages I/II). Clear cell carcinomas are more commonly found at stage I.[29]

Reviewing the articles on gene expression patterns that distinguished the histological subtypes (Table 4), the earlier mentioned article by Lu *et al.*[23] revealed that all serous tumours, 89% of the endometrioid, 43% of the clear cell tumours and 22% of the mucinous, were separated from normal tissue with elevated HE4 using RDPA on mRNA expression levels. A low CA125 separated 78% of the mucinous and 11% of endo-

Table 4 – Microarray studies related to pathological subtype

Study	N	Ovarian tissue	Microarray type	Technical validation	Performance in an independent validation	Details
Schwartz *et al.*[30]	113	53 serous *versus* 10 mucinous *versus* 33 endometrioid *versus* eight clear cell *versus* nine mixed histology	Affymetrix oligonucleotide	1. Three genes (FXYD2, TFF1 and WT1) with qRT-PCR 2. TFF1 and WT1 used for IHC 3. PCA and leave-1-out cross-validation	–	73 genes were higher expressed in clear cell carcinomas compared to other histological subtypes. 88% accuracy with overall overexpressed 158 genes
Lu *et al.*[23]	42	42 OSPC *versus* five pools of NOVA	Affymetrix oligonucleotide	1. RT-PCR 2. Five genes used for IHC	–	Combination of HE4 and CA125 separated most of the four major histological subtypes
Zorn *et al.*[31]	44	24 serous *versus* 11 endometrioid *versus* nine clear cell	cDNA	1. RT-PCR 2. IHC 3. Leave-1-out cross validation	–	53 genes distinguished clear cell carcinomas from other ovarian histological subtypes
Jazaeri *et al.*[34]	61	18 BRCA1 *versus* 16 BRCA2 *versus* 27 sporadic	cDNA	RT-PCR: 6 genes	–	110 genes could separate ovarian tumours into BRCa1-like and BRCA2- like tumours 53 genes were differentially expressed between BRCA1 tumours and sporadic tumours

Abbreviations: (q)RT-PCR, (quantitative) real time-polymerase chain reaction; PCA, principal component analysis; OSPC, ovarian serous papillary carcinoma; NOVA, normal ovarian epithelium; IHC, immunohistochemistry.

metrioid tumours from the remaining clear cell tumours and normal samples. A profile in clear cell tumours was found by Schwartz *et al.*,[30] that was different from other histological subtypes. Using 158 genes, 7 out of 8 (88%) clear cell carcinomas were correctly classified. The overexpression of certain genes like glutathione peroxidase 3, glutaredoxin and superoxide dismutase in clear cell carcinomas may explain their rather poor prognosis compared to other subtypes. These genes are all antioxidants, which implicates that there might be a role for antioxidant inhibitors in the therapy of clear cell carcinomas.[30] More recently, these findings were confirmed.[31] However, on both articles it appears that there is also a certain overlap of genes between the histological subtypes, which implies that at least some part of the carcinogenesis is shared.[30,31] Indeed, all epithelia of the reproductive tract derive from the Müllerian ducts which may explain the overlap.

In conclusion, these results indicate that different histological subtypes might be partly reflected by a different aetiology through the deregulation and activation of different pathways. In addition, this heterogeneity could therefore also lead to different tumour behaviour, which was postulated by Hogg *et al.*[19] The next step to a more definite confirmation, which is required, is an independent validation.

4. Gene expression profiling of patients with hereditary ovarian cancer

Of all ovarian cancer cases 5–10% are associated with germline mutations, primarily in BRCA1 and BRCA2. Serous tumours are two times more frequent in patients with a BRCA1 or BRCA2 mutation than in patients without these mutations. In a systematic review of 178 BRCA1, 29 BRCA2 mutation carriers and 235 controls, both BRCA1 and BRCA2 ovarian carcinomas were also of higher grade than controls. Furthermore, a higher frequency of P53 staining was found in tumours of patients with BRCA1 or BRCA2 mutations compared to tumours of patients with sporadic ovarian tumours.[32] At this moment there are discrepancies in the results of published studies on survival in patients with BRCA associated ovarian cancer,[7,10,33] although they clearly exhibit a number of histopathological and molecular genetic features typically associated with a poorer survival as compared to sporadic ovarian cancers as described above.[32] Genetic analysis should be able to give us more insight into the biology of a BRCA1 ovarian carcinoma. Jazaeri *et al.*[34] revealed that ovarian carcinomas from BRCA1 and BRCA2 germline mutation carriers have different gene expression profiles suggesting different pathways of carcinogenesis (Table 4). A group of 110 genes could separate ovarian sporadic tumours into BRCA1-like and BRCA2-like subgroups. Many of the overexpressed genes involved in BRCA-pathways were interferon inducible; some were members of the major histocompatibility complex class II family. These genes could be used in the future as an immunotherapeutic target.[34] These findings are in agreement with a previous study reporting differences in gene expression profiles between BRCA1 and BRCA2 breast carcinomas.[35] The fact that BRCA1 and BRCA2 breast tumours are so different at the level of gene expression suggests that they perhaps arise from different precursors. Furthermore, also in sporadic breast tumours both BRCA1 and BRCA2 expression patterns were seen. An explanation for this could be a disruption in the BRCA function such as promoter hypermethylation in sporadic tumours. Comparison of these data with a comparative genomic hybridisation (CGH) study of ovarian tumours supports the hypothesis that BRCA1 and BRCA2 tumours develop through a different tumour development pathway. The number of genetic alterations was significantly higher in tumours of both BRCA1 and BRCA2 mutation carriers compared to sporadic tumours.[36]

In conclusion, ovarian cancers with germline mutations in the BRCA1 and BRCA2 genes both have different pathways involved in its carcinogenesis compared to the development of cancer in sporadic cases. Stronger evidence in the sense of validation is needed.

5. Gene expression profiling and prognosis

At this moment, a staging operation (peritoneal washings, total hysterectomy and bilateral salpingo-oophorectomy, omentectomy, biopsies from the peritoneal surface of the diaphragm, both paracolic gutters, the mesentery and the pelvic side-walls and systematic para-aortic and pelvic lymphadenectomy) and a debulking operation (total hysterectomy and bilateral salpingo-oophorectomy, omentectomy and removal of all tumour tissues if possible) are the standard surgical treatment in patients with ovarian cancer.[1] The extent of the disease correlates with surgery outcome and both are important prognostic factors. Patients with stage I disease have a 5-year survival rate of 90%. For patients with advanced stage disease, the 5-year survival rate is much worse. Within each stage, there are short-term and long-term survivors. The need for an improved staging in these subgroups is obvious. Therefore, microarray analysis may be a useful additional tool providing a molecular staging of patients.

In the study by Schridhar *et al.*,[37] seven early (FIGO stages I/II) and seven late stages (III/IV) ovarian tumours were analysed by hierarchical clustering after hybridising these tumours on cDNA microarrays containing 25,000 genes (Table 5). Matrilysin, gelatinase and matrix metalloproteinase 10 and 12, genes involved in invasion and metastasis, were 5-fold upregulated in both early and late stage carcinomas. Similarities in gene expression were found in early and late stage cancers, whereas differences were observed with CGH, suggesting a tumour progression model.[37]

Optimal surgical outcome seems to be an important prognostic factor. Some people argue that the survival benefit has more to do with the resectability than with the resection, i.e. more with the biological characteristics of the tumour than with the surgery itself.[38] In a meta-analysis by Bristow *et al.*,[2] comprising a total of 81 cohorts of patients with stages III–IV ovarian carcinoma, cohorts with <25% maximal cytoreduction had a mean weighted median survival of 22.7 months, whereas cohorts with >75% maximal cytoreduction had a survival of 33.9 months – an increase of 50%.[2] The result of this study is an evidence for the importance of surgical resection. This does not exclude the possibility that resectability as a biological feature also plays a role.

Table 5 – Microarray studies related to clinical outcome in ovarian cancer

Study	N	Prognostic/predictive subject	Microarray type	Technical validation	Performance in an independent validation	Details
Schridhar *et al.*[37]	38	21 stages I/II *versus* 17 stages III/IV	cDNA	1. RT-PCR 2. CGH analysis	–	7 genes with differentially expressed between the two groups, however the CGH did not confirm these data
Berchuck *et al.*[39]	44	19 optimal *versus* 25 sub-optimal debulking	Affymetrix oligonucleotide	Leave-1-out cross validation	–	73% accuracy of debulking status with 32 genes
Selvanayagam *et al.*[47]	8	4 DOD *versus* 4 NED	Nylon membrane cDNA	–	–	The two groups clustered correctly
Spentzos *et al.*[50]	68	Training set *N* = 14:early relapse (<26 months) *versus* late relapse (>58 months)	Affymetrix oligonucleotide	Leave-1-out cross-validation	Independent test set *N* = 34	An 115-gene signature was found which was an independent prognostic determinant after multivariate analysis
Berchuck *et al.*[51]	65	Training set *N* = 54: Overall survival <3 years *versus* overall survival >7 years	Affymetrix oligonucleotide	1. RT-PCR 2. Tree analysis 3. linear discriminant analysis	Independent test set *N* = 11	85% (46/54) accuracy in training set 100% accuracy in test set
Hartmann *et al.*[52]	79	Training set *N* = 51:early relapse (<21 months) *versus* late relapse (>21 months)	Nylon cDNA	–	Independent test set *N* = 28	78% (40/51) accuracy in training set 86% (24/28) accuracy in test set
Jazaeri *et al.*[48]	45	21 chemosensitive *versus* 24 chemoresistant	cDNA	1. Leave-1-out cross-validation 2. IHC	–	78% accuracy with nine genes
Spentzos *et al.*[49]	60	Training set *N* = 24: residual tumours *versus* complete remission after treatment	Affymetrix oligonucleotide	Leave-1-out cross-validation	Independent test set *N* = 36	91% accuracy in training set Classifier remained an independent prognostic determinant after multivariate analysis

Abbreviations. (q)RT-PCR, (quantitative) real time-polymerase chain reaction; DOD, death of disease; NED, no evidence of disease; IHC, immunohistochemistry.

The best available evidence of the use of molecular profiling to predict optimal *versus* suboptimal cytoreduction comes from a single centre study by Berchuck *et al.* (Table 5).[39] Forty-four advanced serous ovarian carcinomas (19 debulked optimally, i.e., residual tumour nodules <1 cm *versus* 25 debulked suboptimally) were analysed with Affymetrix arrays which contained more than 22,000 genes. The hypothesis used is that if differences in gene expression profiling between optimally and suboptimally debulked cancers are found, then the biological differences rather than the extent of cytoreduction are the primary prognostic factors. The tumours could be subdivided into two subgroups after an internal cross-validation with 32 genes and a 72.7% predictive accuracy. Therefore, the authors concluded that these data support their hypothesis that there are biological differences between cancers that are optimally *versus* suboptimally debulked.[39]

However, it is still not clear from this study whether the size of the tumour bulk at diagnosis, the remaining tumour bulk after surgery or the underlying tumour gene profile determines the survival.[39] Furthermore, with respect to the small numbers, the problem that can occur is called overfitting.[40]

6. Microarray analysis to predict chemosensitivity

The recommended standard chemotherapy for advanced ovarian carcinoma comprises a combination of paclitaxel and platinum based postoperative chemotherapy.[1,41] Randomised trials have clearly shown that these women will benefit from platinum based chemotherapy following cytoreductive surgery with respect to survival and time to recurrence as compared to earlier used regimens.[1,42] Chemotherapy also has its limitations. Many women undergo this treatment with both acute and long-term toxicity.[43] More importantly, although standard treatment will result in an initial response rate of more than 70%, only 50% of these patients with an initial response to chemotherapy will still be

alive after 5 years.[42] Platinum resistance is considered multifactorial and includes pharmacokinetic, tumour microenvironmental and cancer cell specific mechanisms such as decreased drug uptake and increased repair of platinum DNA adducts.[14] Recently, intraperitoneal chemotherapy has attracted much attention.[44,45] Compared with conventional intravenous chemotherapy alone, combined intravenous and intraperitoneal chemotherapy offers a survival advantage although at the price of increased toxicity. Therefore, it would be a big step forward if we were able to separate those patients who will benefit from intensified treatment from those that will not. It has been suggested that microarray analysis can be used as a predictor of chemotherapy response and chemoresistance by revealing the underlying biological mechanisms. There are preliminary data on microarray analysis published that look very promising, although sample size is small. In a microarray analysis of 14 cell lines of which seven were from patients who failed chemotherapy, a significant association of Stat I expression was found with a decreased sensitivity to cisplatin ($r = 0.65$) and AMD472 (a platinum-based chemotherapy, $r = 0.76$). These results were later on confirmed with qRT-PCR.[46] No distinction was made between cell lines of patients treated with cisplatinum and patients treated with carboplatin. Furthermore, it needs to be established that the genes found in vitro apply also to chemosensitivity *in vivo*. Selvanayagam *et al.*[47] performed expression arrays on samples of eight patients, four of which were classified as chemosensitive and four of which were chemoresistant (Table 5). Chemoresistance was considered when the patient relapsed or had progressive disease within 6 months. All patients were treated with platinum-based chemotherapy. The top 100 genes predicted the response correctly in all cases, however, they are not yet validated.[47]

In a larger series of samples from high stage patients, 85 genes were differentially expressed between chemosensitive and chemoresistant tumours. The magnitude of the difference ($\leqslant$2-fold), was rather modest. The authors postulate that the reason for this could be that only a small percentage of the cells are likely to possess a chemoresistant phenotype and therefore this will result in a dilution of the observed gene expression differences. Also, it is likely that chemoresistance is partly acquired under treatment exposure. This hypothesis was supported by differences seen between post chemotherapy samples and primary chemosensitive tumours.[48] Recently, these findings have been confirmed in another study that included an independent test group. Limitation of this study was the difference in end-point between the training (chemoresistance) and test (overall and disease free survival) series.[49] Taken together, chemoresistance is likely to be caused by intrinsic pathways as well as by acquired molecular changes.

The use of gene expression profiling may identify different groups of patients as candidates for different treatment approaches based on the likelihood of achieving a specific good or poor survival. Spentzos *et al.*[50] found a profile of 115 genes in a training cohort of 68 patients, whose signature could distinguish patients into those with a favourable survival and those with an unfavourable overall survival (median, 30 months *versus* not yet reached) in independent validation setting with an accuracy of 85% by weighted voting. The signature also remained an independent prognostic factor in a multivariate analysis. Similar patterns distinguishing short-term and long-term survivors were found by Berchuck *et al.*[51] The classifier, existing of 186 genes, predicted 19 of 24 long-term survivors and 27 of 30 short-term survivors, all high stage, correctly (85% accuracy) and achieved a 100% accuracy in an independent test set of 11 stages I/II ovarian cancers. Hence, the observation that in this study early stage ovarian cancers always shared the same expression profiles with good survivors implies that the poor survivors with high stage ovarian cancer even at an earlier stage will not be picked up by screening.[51] These findings provide evidence for the models proposed by Hogg *et al.*,[19] which suggest that cancers diagnosed at an early stage are of different biological origin, are less likely to progress and have a more favourable outcome. In a more recent study, a 14-gene profile found in a training set of high grade advanced stage ovarian tumours predicted the outcome of 24 of 28 test samples correctly with respect to early relapse after platinum-paclitaxel chemotherapy. However, the reported top 14 genes did not list any of the known markers associated with drug resistance like MRP1, Bcl-2 or GSTs.[52]

Summarising, gene expression profiling was found to be a prognostic tool with a predictive performance of 78–86%. In total less than 500 genes were overexpressed in chemoresistant tumours as compared with chemosensitive ovarian tumours. To establish the role of microarray analysis as a predictor of prognosis with respect to chemosensitivity, (randomised) trials are needed. The inherent statistical fault arising from the analysis of more than 10,000 DNA elements stresses the need for an external validation in a much larger series. Finding new genes related to potential mechanisms that underlie platinum resistance is essential in developing novel treatment strategies. Especially the small sample size of these microarray studies hamper, therefore, the incorporation of these findings into the clinic.

7. Discussion and prospects for the future

Current molecular profiling data of ovarian cancer are starting to give us new insights into the genesis of ovarian cancer. Several studies demonstrated that gene expression profiles can be used to derive sets of genes that can distinguish normal ovarian tissue from invasive ovarian carcinomas. Independent validation of studies describing these differences in gene expression between malignant ovarian tumours compared to normal tissue may result in the identification of (a set of) markers valuable for the detection in an early stage, especially needed in the management of high risk women.

Given the published results of the articles reviewed, the models proposed by Hogg *et al.*[19] are to some extent supported by the profiling studies of the histological subtypes. In these models, it is proposed that a difference in tumour behaviour is displayed by a difference in the underlying histological features. For further extension of the models, microarray experiments analysing the correlation between different grades of ovarian cancers and stage distribution will be needed.

High stage patients are usually treated with surgical exploration, tumour debulking and subsequent chemotherapy. For the prediction of surgery outcome, the individual skills of the gynaecologist are of utmost importance. Therefore, the clini-

cal significance of microarray studies predicting the debulking status seems at this point rather low.

Worldwide, the combination of paclitaxel and platinum chemotherapy has been incorporated in the standard protocol for the management of patients with advanced stage ovarian cancer, although the outcome in individual patients is uncertain. It remains to be established if other drug agents achieve any advantages over the standard first line treatment. We assessed the literature on microarray analysis addressing chemosensitivity. The predictive performance of the classifiers found is encouraging with 78–86% accuracy. More knowledge about chemosensitivity will lead to more individualisation of therapy. It could be possible to decrease the amount of chemotherapy prescriptions or in case of a poor prognosis to an intensified treatment by means of intraperitoneal chemotherapy.

However, one should bear in mind that at this moment microarray data from large (prospective) trials are not (yet) available. With increasing numbers of data from published reports, access to these data for the reproducibility of its results and pooling becomes more and more important.

Despite its rapid incorporation in research and although this procedure seems to be very promising at this moment, indistinctness with respect to its role in clinical management remains which should be resolved in the near future.

Conflict of interest statement

None declared.

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