The significance of p53 isoform expression in serous ovarian cancer

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Evaluation of: Hofstetter G, Berger A, Schuster E *et al.* Δ 133p53 is an independent prognostic marker in p53 mutant advanced serous ovarian cancer. *Br. J. Cancer* 105(10), 1593–1599 (2011). Epithelial ovarian cancer still carries a high mortality rate and remains the leading cause of gynecologic cancer death in the USA, despite decades of research. Hence, there is considerable interest in developing biomarkers that could be used to stratify patients for subsequent treatment. Mutation of the p53 tumor suppressor gene occurs very frequently (~96%) in high-grade serous ovarian cancer. However, loss of p53 has not proven to be a reliable prognostic marker. Recent evidence indicates that the truncated p53 protein isoforms can regulate activated p53 and thus may play a role in tumorigenesis. In the article by Hofstetter *et al.*, the authors examined the relationship between the expression of two p53 isoforms (Δ 133p53 and Δ 40p53) and prognosis in patients with serous ovarian cancer. Their findings indicate that Δ 133p53 constitutes an independent prognostic marker for improved recurrence-free and overall survival. Intriguingly, this relationship was observed in patients whose tumors expressed a mutant p53, suggesting that Δ 133p53 might suppress the actions of mutant p53. The mutational status of p53 alone did not have prognostic significance. These studies suggest that mutant p53 activity may be counteracted by Δ 133p53, which leads to a more favorable prognosis in advanced serous ovarian carcinomas. Novel therapeutic approaches could be built upon these findings.

Summary of methods & results

The objective of the studies was to evaluate the clinical relevance of p53 (Δ 133p53, Δ 40p53 and full-length p53 [FLp53]) and p73 isoforms (TAp73 and Δ TAp73) as potential prognostic markers in advanced serous ovarian cancer. The cohort of patients for this study consisted of 154 patients diagnosed with primary advanced serous ovarian cancer, providing a uniform patient population with most (81.8%) presenting with International Federation of Gynecology and Obstetrics (FIGO) stage III disease. A small fraction (18.2%) presented with stage IV disease. Most tumors (72.7%) were poorly differentiated and most (87.8%) underwent platinum-based chemotherapy after surgical debulking of the tumor. The median follow-up was 24.5 months.

A molecular analysis was conducted on tissue samples collected at the time of diagnosis. RNA was isolated from frozen tumor specimens and subjected to real-time PCR after reverse transcription using primer sets for p53 (Δ 40p53, Δ 133p53 and FLp53) and p73 isoforms (TAp73 and Δ TAp73) to quantitate p53 and p73

isoforms. p53 mutational status was determined by sequencing the p53 gene and confirming that p53 was inactive using a functional yeast-based assay. The resulting values for p53 and p73 isoforms were used to fractionate samples into two groups, a high-expressing group and a low-expressing group. The χ^2 -test was used to examine the relationship between isoform expression and clinicopathologic parameters.

The results suggested a significant correlation between Δ 133p53 expression levels and survival. Specifically, on multivariate analysis, Δ 133p53 was an independent prognostic factor for both recurrence-free and overall survival in 121 patients who had p53-mutant ovarian cancer. High Δ 133p53 levels imparted a 43% reduction in the risk of recurrence and a 64% reduction in the risk of death compared with patients whose tumors had low Δ 133p53 levels. The near association between platinum resistance and Δ 133p53 levels in p53-mutant cases, with 34.4% of patients with low Δ 133p53 levels failing to respond to platinum therapy, is intriguing.

Keywords

- biomarker ■ ovarian cancer
- p53 isoforms ■ p73
- prognosis

Expression of the $\Delta 40p53$ isoform on univariate analysis imparted an improved recurrence-free survival in patients whose tumors had wild-type $p53$, and this finding was associated with grade I/II disease. With the exception of this association with tumor grade, in general, $p53$ isoform expression was not correlated with clinicopathologic parameters.

Discussion

Epithelial ovarian cancer still carries a high mortality rate and remains the leading cause of gynecologic cancer death in the USA, despite decades of research. There are more than 22,280 new cases and 15,500 deaths expected in 2012 [101]. There has been no significant improvement in the 10-year survival rate of 20% for advanced-stage epithelial ovarian cancer patients. The majority of deaths (~70%) in epithelial ovarian cancer occur in patients who are diagnosed with advanced-stage high-grade serous adenocarcinoma. A strength of the study by Hofstetter *et al.* is that it is restricted to advanced-stage (III/IV) serous adenocarcinoma; the most common and clinically relevant ovarian cancer subtype [1]. Furthermore, the tissue specimens in the Hofstetter study were accrued through a prospective multicenter project that sampled a wide range of patients and was accompanied by extensive follow-up information. These features of the study make the interpretation of the results more meaningful.

While mutations in the $p53$ gene are a feature of 50% of cancer cases overall, a systematic study by the Cancer Genome Atlas project found that almost all (96%) of serous ovarian cancer cases harbored a $p53$ mutation [2]. This highlights the importance of this tumor suppressor gene in ovarian carcinogenesis. However, in the current study [1], $p53$ mutational status did not show prognostic significance, which is not surprising considering the conflicting evidence in the literature and is in line with reports in several other cancers. This underscores the complexity of $p53$ pathway functionality. It is well established that $p53$ is a transcription factor that responds to stress and activates a cascade of gene expression that can lead to apoptosis or growth arrest. Regulation of $p53$ activity can occur post-translationally through protein–protein interactions. Indeed, it has been suggested that the $p53$ transcription factor is a multisubunit protein complex composed of different amounts of $p53$ and the related $p63$ and $p73$ protein isoforms, which assemble on the

$p53$ response element of $p53$ -inducible genes [3]. It is now known that 12 distinct isoforms of the $p53$ protein can be generated through alternative splicing, alternative initiation of translation and alternative promoter usage, and that at least some of these isoforms can physically interact with FL $p53$. Hence, $p53$ isoforms may regulate the activity of the full-length protein through protein–protein interactions.

In this context, it is significant that Hofstetter and colleagues show, for the first time, that two $p53$ isoforms that are N-terminally truncated ($\Delta 133p53$ and $\Delta 40p53$) have a favorable prognosis in subsets of advanced-stage epithelial ovarian cancer and that the apparent protective effect of $\Delta 133p53$ occurs in tumors with mutant $p53$. The authors suggest that $\Delta 133p53$ may interact with mutant $p53$ as it oligomerizes with wild-type FL $p53$ and, since $\Delta 133p53$ lacks the transactivation domain, it may act as a dominant negative protein to suppress mutant $p53$'s oncogenic activity. However, it should be noted that $\Delta 133p53$ may also have activities that are independent of its association with FL $p53$, such as its ability to stimulate the expression of Bcl2 in response to stress [4]. The fact that several clinical studies showed that $\Delta 133p53$ was abnormally expressed in some tumors lends support to the notion that $\Delta 133p53$ plays a role in tumorigenesis, even though the clinical relevance of its expression in those tumors was not clear [5].

The authors also showed that high levels of $\Delta 40p53$ expression were significantly associated with improved recurrence-free survival; however, an explanation of these results is less obvious. All $\Delta 40p53$ isoforms contain transactivation domain II; hence, these proteins might be expected to have the capacity to activate gene expression, although it is uncertain whether $\Delta 40p53$ can induce the same cohort of genes as the wild-type FL $p53$ [6]. Since $\Delta 40p53$ forms a complex with wild-type FL $p53$, elevated levels of the isoform may stabilize the full-length protein and promote $p53$ -mediated gene expression and its tumor suppressor activity [7].

Collectively, these studies suggest that elevated levels of some $p53$ isoforms may have favorable prognostic significance, which implies that the extent of function of the $p53$ pathway may be determined by the presence of one or more $p53$ isoforms. These results also highlight the complexity of the $p53$ pathway and point out that the $p53$ pathway may be controlled in unexpected ways that influence the pathways that are involved in tumorigenesis.

Future perspective

Identification of biomarkers that could inform a therapeutic strategy is an essential step in developing targeted personalized therapy. In the design of clinical trials that study therapeutic approaches targeting p53, the p53 status (mutation vs wild-type) is frequently used for triaging or classifying patients. In a recent review, the National Cancer Institute (NCI) clinical trials database listed 151 trials that exploit the p53 pathway [8]. However, as this paper by Hofstetter *et al.* demonstrates, the p53 network is complex and mutation status is probably not a sufficient determinant of p53 pathway activity [1]. In this respect, the studies by Hofstetter and colleagues are important because detecting the presence of p53 isoforms may be useful to more accurately stratify patients for clinical trials. These results also have therapeutic implications. Therapeutic approaches could be modified to take into account these new findings. For example, gene therapy approaches to restore wild-type p53 function could deliver a p53 isoform (instead of the wild-type p53 gene) to patients whose tumors harbor an inactivated p53. Activating wild-type p53 and/or inactivating mutant p53 by modulating these p53 isoform regulators are other potential approaches. It is important that a better understanding is gained of interactions within what is clearly a complex p53 network.

These results also have mechanistic implications. Loss of p53 is a common event during tumorigenesis, suggesting that inactivation of this tumor suppressor is a key event during tumorigenesis. However, regulation of the p53 pathway is complex and mutations in the

p53 gene result in the expression of a mutant protein that has oncogenic activity. Hence, the notion that p53 mutations inactivate the p53 pathway is an oversimplification. More recently, the discovery of two other p53 family members, p63 and p73, and of at least a dozen truncated p53 isoforms that can interact with and modify the activity of the full-length protein, suggests that these isoforms play a key role in regulation of p53. Several studies have shown that $\Delta 133p53$ proteins are present at elevated levels in the cells of some tumor types, suggesting that they may play a role in tumorigenesis [9]. The results by Hofstetter and colleagues support this notion and suggest that the $\Delta 133p53$ and $\Delta 40p53$ isoforms may either substitute for the wild-type p53 protein or suppress the actions of mutant p53 and ameliorate the loss of p53 tumor suppressor activity. Hence, these two isoforms may have modest tumor suppressor activity in their own right. Further studies in other tumors and of other isoforms will be necessary to elucidate the full implications of p53 isoforms and their role in a malfunctioning p53 pathway in human tumors.

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Executive summary

Study design

- A total of 154 patients with primary advanced serous ovarian cancer were enrolled in the study.
- The mutational status of p53 in resected tumors was determined by sequencing and a yeast-based functional assay.
- The levels of several p53 ($\Delta 40p53$, $\Delta 133p53$ and full-length p53) and p73 (TAp73 and Δ TAp73) isoforms were determined using real-time PCR.
- Patients were followed for a median of 24.5 months (range: 1–49 months).

Results

- Elevated expression of $\Delta 133p53$ correlated with improved recurrence-free and overall survival in patients with mutant p53.
- Elevated expression of $\Delta 40p53$ was associated with improved recurrence-free survival in patients with wild-type p53.
- There was no prognostic significance associated with the p73 isoforms.

Conclusion

- This study suggests that elevated expression of some p53 isoforms can indicate a favorable prognosis; however, this is determined by the context (p53 mutational status) in which these isoforms are expressed.
- These results suggest that p53 isoforms may be useful prognostic markers and have mechanistic implications for regulation of the p53 pathway.

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