Expression of transcription factor AP-2α predicts survival in epithelial ovarian cancer

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Summary The 52-kDa activator protein (AP)-2 is a DNA-binding transcription factor which has been reported to have growth inhibitory effects in cancer cell lines and in human tumours. In this study the expression of AP-2α was analysed in 303 epithelial ovarian carcinomas by immunohistochemistry (IHC) with a polyclonal AP-2α antibody and its mRNA status was determined by in situ hybridization (ISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). The immunohistochemical expression of AP-2α was correlated with clinicopathological variables, p21/WAF1 protein expression and survival. In normal ovaries, epithelial cells expressed AP-2α protein only in the cytoplasm. In carcinomas nuclear AP-2α expression was observed in 28% of the cases although cytoplasmic expression was more common (51%). The expression of AP-2α varied according to the histological subtype and differentiation. AP-2α and p21/WAF1 expressions did not correlate with each other. Both in univariate (P = 0.002) and multivariate analyses (relative risks (RR) 1.6, 95% confidence interval (CI) 1.13–2.18, P = 0.007) the high cytoplasmic AP-2α expression favoured the overall survival. In contrast, the nuclear AP-2α expression combined with low cytoplasmic expression increased the risk of dying of ovarian cancer (RR = 2.10, 95% CI 1.13–3.83, P = 0.018). The shift in the expression pattern of AP-2α (nuclear vs cytoplasmic) in carcinomas points out to the possibility that this transcription factor may be used by oncogenes in certain histological subtypes. Based on the mRNA analyses, the incomplete expression and translation of AP-2α in ovarian cancer may be due to post-transcriptional regulation. © 2000 Cancer Research Campaign

Keywords: epithelial ovarian cancer; prognosis; AP-2α; p21/WAF1

The 52-kDa activator protein (AP)-2 is a DNA-binding transcription factor, which was originally purified from HeLa cells and identified as a nuclear factor, regulating the expression of human metallothionein IIa (Lee et al, 1987; Williams et al, 1988). AP-2 also mediates programmed gene expression during embryogenesis including genital tract, and cell differentiation in adult tissues (Williams et al, 1988; Mitchell et al, 1991). The AP-2 amino acid sequence is highly conserved in mice and humans indicating that this protein plays a fundamental role in development (Winning et al, 1991). Transcriptionally AP-2 functions as a dimer and recognizes GC-rich DNA-sequences (Williams et al, 1988). The DNA-binding domain is located within the C-terminal half of the protein, showing high conservation (75–80%) within three human AP-2 related genes AP-2α, -β and γ (Bosher et al, 1996; Williamson et al, 1996).

In normal cells, AP-2 regulates gene expression in response to different signal transduction pathways. Signals mediated through the activation of cAMP-dependent protein kinase and protein kinase C induce AP-2 activity independent of protein synthesis (Chiu et al, 1987; Imagawa et al, 1987; Hyman et al, 1989). In contrast, retinoic acid induces AP-2 activity by increasing AP-2 mRNA levels (Williams et al, 1988; Luscher et al, 1989). AP-2 activates both in vivo and in vitro transcription of promoters containing AP-2 binding sites, which have been identified in the enhancer regions of viral genes (Mitchell et al, 1987), proenkephalin, keratin K14 (Leask et al, 1991), c-erbB-2 (Bosher et al, 1995), plasmogen activator type I, c-Myc (Imagawa et al, 1987), E-cadherin (Hennig et al, 1996), type IV collagenase (MMP-2) (Huhtala et al, 1990) and p21/WAF1 (Zeng et al, 1997). Recently, it was reported that transcriptional stimulation of E-cadherin promoter by pRb and Myc is mediated by AP-2 in epithelial cells (Batsche et al, 1998).

The AP-2α, -β and -γ genes are located at chromosomes 6p24, 6p12 and 20q13 respectively (Williamson et al, 1996). Loss of heterozygosity has been frequently reported on chromosome 6p in human cancers, including ovarian cancer (Sato et al, 1991; Foulkes et al, 1993). In cancer cell lines (Bar-Eli, 1997; Zeng et al, 1997; Jean et al, 1998) and in malignant melanoma (Karjalainen et al, 1998) AP-2 has been shown to possess or exert growth inhibitory effects. The tumour suppressive effects of AP-2 are, at least in part, mediated through p21/WAF1 activation (Zeng et al, 1997). To explore the significance of AP-2 in ovarian cancer, we studied the expression of AP-2α in 303 epithelial ovarian carcinomas using IHC. Furthermore, the relationships between AP-2α and p21/WAF1 expressions, histopathological variables and patient survival were analysed. To further elucidate the regulatory mechanisms, the mRNA status of AP-2α was studied by RNA ISH and confirmed by RT-PCR.

MATERIALS AND METHODS

Patients

The material of the present study was selected from a consecutive series of 445 women diagnosed and treated for ovarian malignancy
at Kuopio University Hospital and Jyväskylä Central Hospital, Finland, between 1976 and 1992 and subsequently followed-up until September 1996. The non-epithelial type of neoplasia and all patients treated before operation or unoperated patients were excluded. Depending on the availability of representative tumour samples, a total of 303 patients were included in the analyses.

All tumours were staged according to the International Federation of Gynecology and Obstetrics (FIGO) standards (Cancer Committee of the International Federation of Gynecology and Obstetrics, 1989). Retrospective review of the patient files was performed to obtain all pertinent data on the primary tumour, type of surgery, adjuvant treatment, recurrence and survival. Patients who died because of any post-operative complications were excluded from the survival analyses (n = 9). The clinicopathological data of the patients are summarized in Table 1.

Of the 303 patients, 120 underwent radical surgery (i.e. no primary residual tumour), 221 received post-operative chemotherapy, nine received post-operative radiotherapy and 38 women received both therapies. Disease recurrence was observed in 73 patients (24%), no recurrence in 92 patients (30%) and in 138 patients (46%) the tumour was present or progressing. The median follow-up time for all patients (n = 303) was 27 months (range 1–237 months) and for patients still alive (n = 77) 106 months (range 22–237 months).

| Table 1 Clinical data of the patients |
|---------------------------------------|
| Age at diagnosis, years               | Median 62 | 18–85 |
| Histological type                     | epithelial | n %   |
|                                      | serous      | 107 35|
|                                      | mucinous    | 30 10 |
|                                      | endometrioid| 82 27 |
|                                      | clear cell  | 31 10 |
|                                      | other†      | 53 18 |
| Histological grade                   | 1           | 41 13 |
|                                      | 2           | 105 35|
|                                      | 3           | 157 52|
| FIGO stage                           | I           | 83 27 |
|                                      | II          | 46 15 |
|                                      | III         | 144 48|
|                                      | IV          | 30 10 |
| Primary residual tumour              | no data     | 26 8 |
|                                      | none        | 120 40|
|                                      | ≤ 2 cm      | 51 17 |
|                                      | > 2 cm      | 106 35|
| Adjuvant chemotherapy                | platinum-containing | 160 53|
|                                      | no platinum-containing | 96 32|
|                                      | none        | 44 15 |
| Chemotherapy response†               | no data     | 7 2 |
|                                      | CR          | 138 45|
|                                      | PR          | 39 13 |
|                                      | SD          | 21 7 |
| Cause of death                       | ovarian cancer | 195 65|
|                                      | other cause | 31 10 |
|                                      | alive       | 77 25 |

†includes 20 mixed epithelial, one Brenner, 32 unclassified epithelial.

Histology

The primary tumour samples were fixed in 10% formalin and embedded in paraffin. From each sample, 5-μm-thick sections were stained with haematoxylin and eosin. Histological typing and grading were done according to the WHO classification (Seren et al, 1973). All tumours were graded as either well, moderately or poorly differentiated by one pathologist (KS) in a blinded manner, i.e. being unaware of the other data.

Immunohistochemistry

AP-2α

Five-micrometre-thick section tumours were deparaffinized and rehydrated using xylene and graded alcohols. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide for 5 min, followed by a wash for 2 × 5 min with phosphate-buffered saline (PBS). The sections were heated in a microwave oven in 10 mM citrate buffer (pH 6.0) for 2 × 5 min and after that allowed to cool for 20 min and washed for 3 × 5 min with PBS. Non-specific binding was blocked with 1.5% normal goat serum in PBS for 30 min at 37°C. The primary antibody, a rabbit polyclonal AP-2α (SC-184) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibody, raised against a peptide corresponding to amino acids 420–437 mapping at the carboxy terminus of AP-2 of human origin, was diluted to 1:2500 and incubated on the slides overnight at 4°C. The slides were incubated with the secondary antibody for 30 min and washed for 2 × 5 min with PBS. The slides were then incubated for 40 min in preformed avidin–biotinylated peroxidase complex (ABC, Vectastain Elite kit, Vector Laboratories, Burlingame, CA, USA) and washed twice for 5 min with PBS, developed with diaminobenzidine tetrahydrochloride (DAB) substrate (Sigma, St Louis, MO, USA), counterstained with Mayer’s haematoxylin, dehydrated, cleared and mounted with DePex (BDH, Poole, UK). A corresponding section processed without the primary antibody was used as a negative control and the inflammatory cells within the tumour served as positive internal controls.

p21/WAF1

The p21/WAF1 protein was demonstrated using a similar IHC staining protocol. We used p21-specific mouse monoclonal antibody (NCL-WAF-1, Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) at a working dilution of 1:10. In each batch, known p21-positive tumour samples were used as positive controls, and the same biopsy processed without the primary antibody was used as a negative control.

Scoring of the AP-2 and p21/WAF1 immunostainings

All slides were evaluated with a microscope (field diameter 490 μm) and the fraction of positive cancer cells was assessed as the percentage of the whole tumour area by two observers (MA, VK) being unaware of the clinical outcome of the patients. Discrepancies between the observers were found in fewer than 10% of the slides examined, and consensus was reached on further review. For AP-2α, both the nuclear and cytoplasmic positivity was recorded. The nuclear AP-2α expression was graded into two groups (negative or positive) according to 5% cut-off point, which was the 75th percentile in a frequency distribution. The cytoplasmic AP-2α expression was graded into two groups according to 50th percentile in a frequency distribution:
low, when < 10% of tumour cells expressed AP-2α and high, when ≥ 10% of tumour cells expressed AP-2α. For p21, the positivity was assessed as the percentage of positively stained tumour cell nuclei. For further analyses, p21/WAF1 immunoreactivity was categorized into two groups: (1) low expression, if < 10% of the tumour cell nuclei were positive, and (2) high expression, if ≥ 10% of the tumour cell nuclei were positive (Ito et al, 1996; Wakasugi et al, 1997). The 10% cut-off point was the 75th percentile of the p21/WAF1 expression in a frequency distribution.

mRNA in situ hybridization

A series of 19 samples (both AP-2α-negative and -positive by IHC) being representative of the tumour material were selected for ISH. Deparaffinized 5-μm sections were microwaved in 0.01 M citrate buffer (pH 6.0) for 5 min. The samples were fixed in 4% paraformaldehyde, rinsed, pretreated with 0.1 M hydrochloric acid (HCl) and PBS, followed by digestion for 20 min with proteinase K (Boehringer Mannheim, Mannheim, Germany) at the concentration of 20 μg ml⁻¹. After a short fixation by 4% paraformaldehyde, the sections were rinsed in PBS and in 0.85% sodium chloride (NaCl), dehydrated in graded ammonium acetate alcohols and air-dried. All specimens were then prehybridized for 2 h at 62°C. Recombinant plasmid pHAP2-Hsma containing human AP-2α exon 2 (transactivation domain) in a pBluescript KS+ -vector was linearized to generate antisense- and sense-digoxigenin-labelled probes by DIG RNA labelling kit (Boehringer Mannheim). Probes were added to the hybridization buffer at 5 ng μl⁻¹, and the sections were hybridized overnight at 62°C in a humidified chamber. Subsequently, the sections were washed with increasing stringency. Non-specific binding was blocked with 2% normal sheep serum (Sigma) in 0.1% Triton X/TBS-buffer. The hybridized probe was detected by incubation in anti-digoxigenin alkaline phosphatase conjugate (Boehringer Mannheim) followed by NBT/BCIP. The reaction was stopped with diluted water, sections were dehydrated and coverslipped with Mountex (Histolab Production AB, Göteborg, Sweden). The hybridized probe was detected by incubation in anti-digoxigenin alkaline phosphatase conjugate (Boehringer Mannheim) followed by NBT/BCIP. The reaction was stopped with diluted water, sections were dehydrated and coverslipped with Mountex (Histolab Production AB, Göteborg, Sweden). The specificity of the hybridization signals was demonstrated with digestion of the serial section with RNase before microwave or proteinase K pretreatment to degrade RNA and secondly, a digestion of the serial section with RNAase before microwave or proteinase K pretreatment to degrade RNA. The high cytoplasmic AP-2α expression associated with better differentiation (χ² = 6.1, P = 0.01), absence of residual tumour (χ² = 5.7, P = 0.02) and complete chemotherapy response group (χ² = 7.8, P = 0.05). The nuclear AP-2α expression had no statistical correlation with the p21/WAF1 expression (Figure 1F).

RESULTS

AP-2α expression

In immunohistochemical analyses of AP-2α protein, negative control section remained negative and in tumour sections, strongly positive inflammatory cells served as internal positive controls (Figure 1A). In normal ovarian surface epithelium (n = 19) no nuclear AP-2α expression was observed but high cytoplasmic AP-2α expression was observed in 63% of the cases.

Positive nuclear AP-2α expression was observed in 28% of the tumours (Figure 1B). Of the total (n = 303), 34% of serous, 34% of endometrioid and 42% of clear cell histological subtypes were AP-2α positive (χ² = 22.7, P = 0.0001). The majority of the AP-2α positivity was expressed in the cytoplasm (Figure 1C,D). Of the tumours, 51% were high expressers of cytoplasmic AP-2α including 70% of the mucinous and 65% of the clear cell tumours (χ² = 11.6, P = 0.02). Both nuclear and cytoplasmic AP-2α positivities were observed in 19% of the tumours (Figure 1E).

RT-PCR amplification

To confirm the specificity of the AP-2α mRNA ISH signal, total cellular RNA was extracted from five 20-μm-thick paraffin-embedded tissue sections of the tumour as described previously (Sugg et al, 1998). The RNA was used as a template to synthesize first-strand cDNA, and PCR amplification was performed using the commercially available Enhanced Avian RT-PCR kit (Sigma). The primers for the AP-2α were designed to anneal to exon 2 of the TAF2α gene and their specificity was checked by searching the Blast database. The primers used were: AP-2 U 5'-GCCCCCGTGTCCCTGTCCAA-3' and AP-2 L 5'-TGAGGAGCAGAGGCCGACC-3'. PCR products were electrophoresed on a 6% poly-acrylamide gel and stained with silver nitrate.

Statistical analyses

The SPSS-Win 7.5 program package was used in a PC computer for basic statistical calculations. First, the relationships (Spearman correlations) between AP-2α and p21 expression levels were analysed. Each parameter in the analysis was considered as a continuous variable. The interrelationships between the categorical IHC variables and their associations with clinicopathological parameters were examined by contingency tables which were further analysed by χ²-tests. Univariate survival analyses were based on the Kaplan–Meier method (log–rank analysis) (Kaplan and Meier, 1958). Univariate and multivariate survival analyses were calculated with Cox model using the Log likelihood ratio significance test in a forward stepwise manner (Cox, 1999). Overall survival was defined as the time interval between the date of surgery and the date of death due to ovarian cancer. Recurrence-free survival was defined by the time interval between the date of surgery and the date of diagnosed recurrence. Probability values less than 0.05 were regarded as significant. In Cox’s multivariate analysis, a removal limit of P < 0.10 was used as an additional criteria.

p21/WAF1 expression

A total of 298 ovarian tumours were evaluable for p21/WAF1 immunostaining which was confined to the tumour cell nuclei. Of
these, 206 tumours (69%) showed low expression of p21/WAF1. The low p21/WAF1 expression associated significantly with the serous and other histological types ($\chi^2 = 41.2, P < 0.000005$), poor differentiation ($\chi^2 = 14.2, P = 0.0001$), advanced FIGO stage ($\chi^2 = 9.0, P = 0.003$) and residual tumour $> 2$ cm ($\chi^2 = 13.1, P = 0.0003$).

**Univariate survival analysis**

Overall disease-related 5-year survival rate of the patients was 37%. When nuclear and cytoplasmic AP-2α expressions were considered separately, nuclear AP-2α expression was not related...
Figure 2  (A) Positive mRNA in situ hybridization analysis for AP-2α and (B) a consecutive negative control section hybridized with the sense probe showing no positivity. Some of the tissue boundaries are marked by arrows.
to survival \((P = 0.45)\) but the high cytoplasmic AP-2\(\alpha\) expression predicted significantly favourable overall survival in univariate analysis \((28\% \text{ vs } 46\%, P = 0.002)\) (Figure 4). When both the nuclear and cytoplasmic AP-2\(\alpha\) expressions were taken into account for univariate survival, the significant survival advantage was related to high cytoplasmic AP-2\(\alpha\) expression without nuclear AP-2\(\alpha\) \((P = 0.006)\). Results of Cox’s univariate survival analysis according to the AP-2\(\alpha\) expression are presented in Table 3. Other significant predictors of favourable overall survival were early FIGO stage \((P < 0.00005)\), differentiation \((P < 0.00005)\), absence of primary residual tumour \((P < 0.00005)\), younger age at diagnosis \((P = 0.0004)\) and high p21/WAF1 expression \((P = 0.02)\).

The high cytoplasmic AP-2\(\alpha\) expression associated significantly with better overall survival also in the subgroups of endometrioid \((P = 0.008)\), clear cell \((P = 0.045)\) and other \((P = 0.03)\) histological subtypes. The same association was observed in moderately \((P = 0.03)\) and poorly \((P = 0.007)\) differentiated tumours. For those patients with advanced FIGO stages high cytoplasmic AP-2\(\alpha\) expression predicted better overall survival \((P = 0.01)\).
Short recurrence-free survival was significantly predicted by serous histological type \((P = 0.01)\), advanced FIGO stage \((P < 0.00005)\) and primary residual tumour > 2 cm \((P < 0.00005)\). AP-2α expression did not relate to the recurrence-free survival in Kaplan–Meier or in Cox’s univariate survival analyses.

### Multivariate survival analysis

The histological type and grade, FIGO stage, age at diagnosis, primary residual tumour, adjuvant chemotherapy, p21/WAF1 and cytoplasmic AP-2α expression were included in the Cox’s multivariate analysis (Table 4). The independent prognostic factors of overall survival were FIGO stage \((P = 0.0009)\), primary residual tumour \((P = 0.0001)\), age at diagnosis \((P = 0.009)\), adjuvant chemotherapy \((P = 0.007)\) and cytoplasmic AP-2 expression \((P = 0.007)\). When combined nuclear and cytoplasmic AP-2α expression was included in the Cox analysis, the risk to die of ovarian cancer was even more increased if nuclear positivity was observed together with low cytoplasmic expression of AP-2α \((RR = 2.10, 95\% \text{ CI} 1.13–3.83, P = 0.018)\). Only the primary residual tumour predicted independently recurrence-free survival \((P < 0.00005)\).

### DISCUSSION

In this study we show that malignant epithelial ovarian tumours express transcription factor AP-2α in cytoplasm as well as in tumour cell nuclei compared to normal ovaries where only cytoplasmic staining was observed. The significance of AP-2α expression in survival analyses points out the importance of this transcription factor in epithelial ovarian malignancy, although the precise mechanisms of AP-2α’s functions remain unknown.

In vitro, AP-2 is expressed in a cell-type-specific manner (Imagawa et al, 1987; Williams et al, 1988). So far, only few studies have examined the expression of AP-2 in human tumours (Gilbertson et al, 1997; Karjalainen et al, 1998; Turner et al, 1998). In the present series, AP-2α expression was observed both in the cancer cell nuclei and cytoplasm, and the expression varied according to the histological subtype and differentiation. Both nuclear and cytoplasmic expression of AP-2α has been observed also in malignant melanomas (Karjalainen et al, 1998), childhood medulloblastomas (Gilbertson et al, 1997), colorectal adenocarcinomas (Ropponen et al, 1999) and in cervical intra-epithelial neoplasms (Hietala et al, 1997). In breast cancer, AP-2α expression was limited to cancer cell nuclei (Turner et al, 1998). Therefore, the pattern of AP-2α expression seems to be tissue type-specific. Other transcription factors such as p53 (Sun et al, 1992, 1995; Bosari et al, 1994; Goldman et al, 1996), BRCA1 (Chen et al, 1995), the C/EBP (CCAAT/enhancer binding protein) (Sundfeldt et al, 1999) and transforming growth factor (TGF)-β inducible early gene (TIEG) (Subramaniam et al, 1998) have also been shown to be expressed in the cancer cell cytoplasm. The precise location of a transcription factor may depend e.g. upon its position in the cell cycle or the phosphorylation status of the factor.
remained positive for AP-2 and what regulates its translocation to the nucleus. The present study showed the expression of AP-2 to be mostly cytoplasmic but failed to answer why ovarian carcinomas produce this particular transcription factor and what regulates its translocation to the nucleus.

The observed amount of immunohistochemical AP-2 positivity was rather low in the present series. However, even after a very thorough adjustment of the staining conditions inflammatory cells remained positive for AP-2 in tumour sections throughout the staining series verifying the quality of our IHC procedure. We performed mRNA ISH in a subgroup of different tumour types to further elucidate the mechanisms of AP-2 expression. Previously, it has been suggested that the expression depletion may originate from a chromosomal deletion of AP-2 coding gene (Jean et al, 1998) or post-transcriptional or -translational regulation (Mitchell et al, 1987; Williams et al, 1988). The mRNA for the transactivating domain of AP-2 was actively produced in all cases, yet in 7/19 cases very little protein was detectable by IHC. This result was additionally confirmed by RT-PCR. Changes in cell signalling pathways have been shown to modify protein synthesis (Kleijn et al, 1998), but presently very little is known about the regulation of AP-2 synthesis. However, our results suggest that post-transcriptional regulation may also be one mechanism which controls the expression of AP-2 in ovarian carcinomas.

Based on the previous studies in malignant melanoma (Bar-Eli, 1997; Jean et al, 1998; Karjalainen et al, 1998) and colorectal cancer cell lines (Zeng et al, 1997) AP-2 has been suggested to be a new tumour suppressor gene. The high cytoplasmic AP-2 protein expression without nuclear positivity favoured the survival of epithelial ovarian cancer patients. Similarly, in endometrial cancer, favourable survival associated with cytoplasmic p53 over-expression (Soong et al, 1996). In that and other studies as well, the cytoplasmic expression of p53 has represented mainly functional wild-type protein (Moll et al, 1992; Bosari et al, 1994; Goldman et al, 1996; Soong et al, 1996). Because it is generally accepted that the nuclear location of a transcription factor is essential for its function, the mechanisms by which the cytoplasmic AP-2 expression exerts its prognostic significance in epithelial cancer remain unknown. One possible pathway which may mediate AP-2's growth inhibitory effects is through p21/WAF1 activation documented in human hepatoblastoma and colon adenocarcinoma cells (Zeng et al, 1997). The expressions of nuclear AP-2 and p21/WAF1 did not mutually correlate in the present study suggesting that p21/WAF1 may not be activated by AP-2 in epithelial ovarian cancer. This is supported by our previous findings which suggested that p21/WAF1 expression is mostly p53-dependent in epithelial ovarian cancer (Anttila et al, 1999).

Interestingly, in survival analyses the combination of nuclear and low cytoplasmic AP-2 expression associated with a significant risk of dying due to ovarian cancer. It may be possible that as the malignant growth gets beyond a certain point or histological type (e.g. serous) there is a shift to a type of expression where AP-2 is needed to activate oncogenes and/or growth factors. Indeed, previous studies have suggested that AP-2 regulates the proto-oncogene c-erbB-2 in breast cancer (Bosher et al, 1996) and that it has a role in the function of Ras oncprotein (Kannan et al, 1994). Similarly, in cervical cancer HPV-16 needs AP-2 to actively produce its own oncogenes E6 and E7 (Bossler AD et al, manuscript

### Table 4

| Category                                | Beta (S.E) | Relative risk (95% CI)   | P-value |
|-----------------------------------------|------------|--------------------------|---------|
| Overall survival                        |            |                         |         |
| FIGO stage                              |            |                         |         |
| I                                      | 0.53 (0.35) | 1.7 (0.86–3.37)         | 0.12    |
| II                                     | 1.03 (0.38) | 2.8 (1.34–5.8)          | 0.006   |
| IV                                     | 1.66 (0.43) | 5.3 (2.27–12.3)         | 0.0001  |
| Age at diagnosis (years)                |            |                         |         |
| <50                                     | *          |                         | 0.009   |
| 50–65                                   | 0.53 (0.24) | 1.7 (1.05–2.73)         | 0.03    |
| >65                                     | 0.75 (0.25) | 2.13 (1.32–3.44)        | 0.002   |
| Primary residual tumour                 |            |                         |         |
| None                                    | *          |                         | 0.0001  |
| ≤2 cm                                   | 1.10 (0.33) | 2.92 (1.56–5.44)        | 0.0008  |
| >2 cm                                   | 1.39 (0.32) | 4.03 (2.16–7.54)        | <0.00005|
| Adjuvant chemotherapy                   |            |                         |         |
| Platinum-containing                     | *          |                         | 0.007   |
| No-platinum containing                  | 0.40 (0.18) | 1.5 (1.04–2.13)         | 0.029   |
| None                                    | 0.91 (0.32) | 2.48 (1.32–4.64)        | 0.0046  |
| Cytoplasmic AP-2α expression            |            |                         |         |
| Low                                     | 0.45 (0.17) | 1.6 (1.13–2.18)         | 0.007   |
| High                                    | *          |                         |         |
| Recurrence-free survival                |            |                         |         |
| Primary residual tumour                 |            |                         |         |
| None                                    | *          |                         | <0.00005|
| ≤2 cm                                   | 1.03 (0.31) | 2.79 (1.52–5.13)        | 0.0009  |
| >2 cm                                   | 1.57 (0.32) | 4.8 (2.56–9.04)         | <0.00005|

*Reference category. CI = confidence interval.
in preparation). Summarizing the available information it is obvious that further studies are needed to extend our knowledge on the regulation of AP-2α expression as well as its function in cancer.

To conclude, AP-2α is expressed in epithelial ovarian carcinoma and its expression is associated with survival of the patients. In particular, the cytoplasmic expression seems to favour a better prognosis. To our surprise, the translational location of AP-2α to its assumed functional place, the nucleus, clearly worsened patients’ survival. Whether this is an indication of a cooperation between AP-2α and an oncogene remains to be seen. Finally, our mRNA results suggest that post-transcriptional events may be important in the regulation of AP-2α, but the precise mechanisms mediating AP-2α’s effects will still need further exploration in the future.

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