available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Short Communication

Role of the tumour necrosis family ligand APRIL in solid tumour development: Retrospective studies in bladder, ovarian and head and neck carcinomas

P. Mhawech-Fauceglia[a,h], *A. Allal*[b,h], *K. Odunsi*[c], *C. Andrews*[d], *F.R. Herrmann*[e], *B. Huard*[f,g,*]

[a]*Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY, USA*
[b]*Department of Radio-Oncology, University Hospital, Geneva, Switzerland*
[c]*Department of Gynecologic–Oncologic Surgery, Roswell Park Cancer Institute, Buffalo, NY, USA*
[d]*Department of Biostatistics, University Hospital, Buffalo, NY, USA*
[e]*Department of Rehabilitation and Geriatrics, University Hospital, Geneva, Switzerland*
[f]*Division of Hematology, University Hospital, Geneva, Switzerland*
[g]*Department of Pathology–Immunology, University Medical Center, Rue Michel Servet 1, 1211 Geneva 4, Switzerland*

ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form
23 June 2008
Accepted 1 July 2008
Available online 19 August 2008

Keywords:
Inflammation
Carcinoma
Proteoglycan

ABSTRACT

A proliferation inducing ligand (APRIL) from the tumour necrosis family (TNF) promotes the natural development of solid tumours in pre-clinical models. Here, we studied the role of APRIL in patients with urothelial bladder, epithelial surface ovarian, and head and neck squamous carcinomas. By using immunohistochemistry, we revealed an upregulation of APRIL expression in lesions from a significant subset of patients compared to corresponding healthy tissues. APRIL upregulation was not due to autocrine production by tumour cells, but rather originated from infiltration of APRIL-producing neutrophils. Heparan sulphate proteoglycan (HSPG) efficiently concentrated secreted APRIL in lesions. Despite this retention, *in situ* APRIL upregulation did not significantly alter disease-free and overall survivals of carcinoma patients in retrospective studies. This indicates that APRIL is not potent enough to promote the development of solid tumour cells under the pressure of chemotherapy.

1. Introduction

A proliferation inducing ligand (APRIL, TNFSF13) from the tumour necrosis family (TNF) was originally found in cell lines and primary samples from various tumour lesions.[1] APRIL mediates a survival/proliferation signal to lymphoma cells, own to their expression of the APRIL-signalling receptors, the transmembrane activator, calcium modulator and cyclophilin ligand interactor (TACI, TNFRSF13) and the B-cell maturation antigen (BCMA, TNFRSF17).[2] This lymphoma-promoting role was recently substantiated clinically with a worst prognosis for patients harbouring high level of APRIL expression in chronic lymphocytic leukaemia[3] and diffuse large B-cell lymphoma.[4]

* *Corresponding author:* Address: Department of Pathology–Immunology, University Medical Center, Rue Michel Servet 1, 1211 Geneva 4, Switzerland. Tel.: +41 22 379 58 11; fax: +41 22 379 58 02.
E-mail address: bertrand.huard@medecine.unige.ch (B. Huard).
[h] Equal contribution.
0959-8049/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2008.07.007

APRIL has also been implicated in the promotion of solid tumour development in *in vitro* and *in vivo* pre-clinical models.[5–7] At first, this promoting role for APRIL on solid tumour cells was not well understood, since TACI and BCMA expression is restricted to hematopoietic cells.[8] Recently, heparan sulphate side chains of proteoglycans (HSPG) were identified as APRIL-binding partners,[9,10] demonstrating the presence of an APRIL receptor on solid tumour cells with multiple roles in tumourigenesis.[11] Here, we present the first retrospective clinical study on the role of APRIL in carcinoma patients receiving standard chemotherapy. We selected widely different solid tumour types such as urothelial bladder, surface epithelial ovarian and head and neck squamous cell carcinomas to broaden our study.

2. Patients and methods

Patients, treatments and tumour characteristics are summarised in Table 1S. Projects were all reviewed and approved by Local Ethics Committees. Patients with a first time diagnosis of carcinoma were included in the study. All tumours were reviewed prior to analyses to confirm histological grade and stage. We performed immunohistochemistry with a pair of APRIL-specific antibodies, Stalk-1 and Aprily-2, on tumour micro-arrays as previously described.[4] Aprily-2 immunoexpression was scored [0] when negative, [1] when focal, [2] when spread and [3] when intense spread stainings were observed. Immunostaining for Stalk-1 was scored as [0] for 0 –5, [1] for 5–15, [2] for 15–25 and [3] for >25 positive cells per sections. A mean >1.5 of Aprily-2 and Stalk-1 samples stained in triplicates was considered as APRIL^{high}. Overall survival (OS) was measured from the date of biopsy until the date of death, excluding co-morbidity. Disease-free survival (DFS) was defined as the time between disease remission and the observed recurrence of the disease. Disease remission was defined as absence of disease at least one month after the last treatment ended. Physical examination, serum testing and imaging studies evaluated absence of disease. OS and DFS rates were

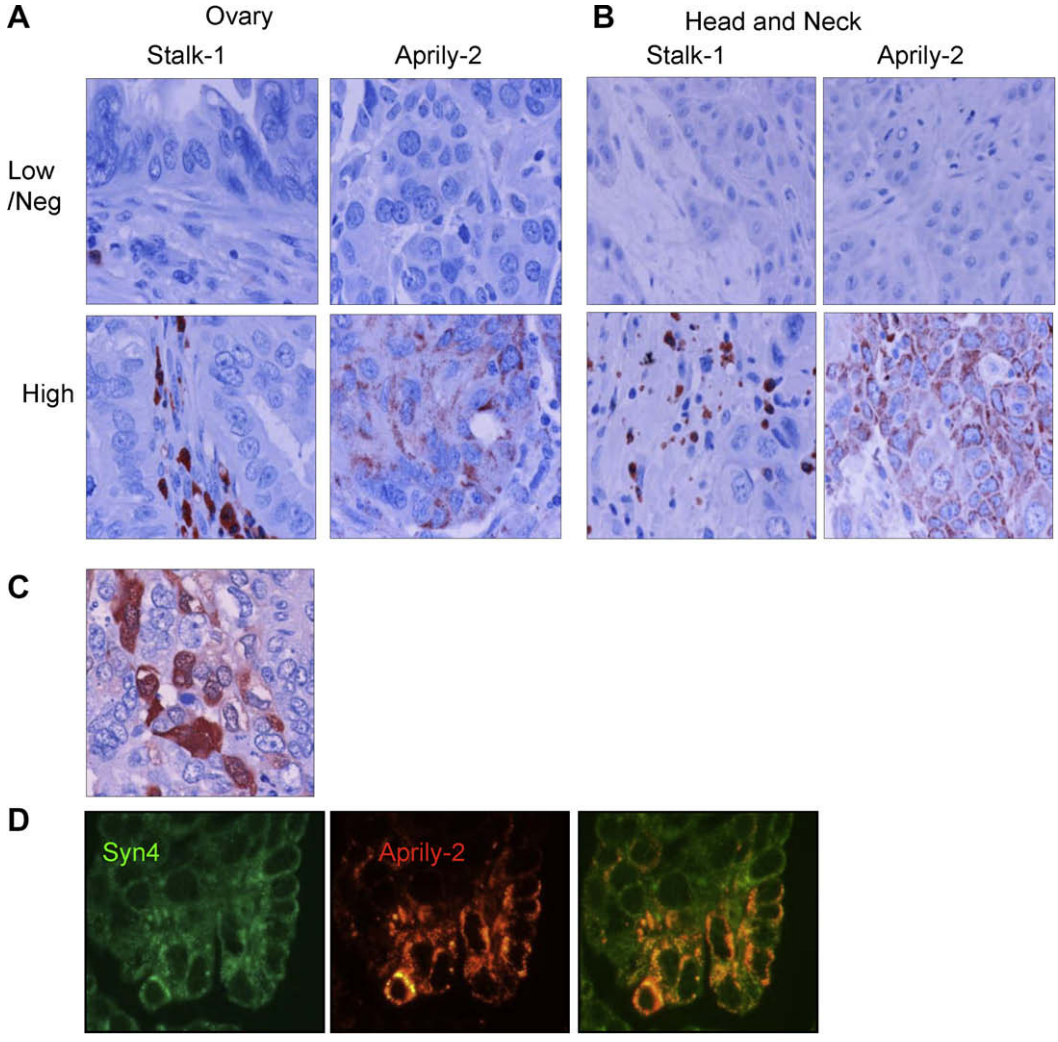

Fig. 1 – APRIL expression in carcinoma patients. Carcinoma lesions were stained with the Stalk-1 and Aprily-2 antibodies. Relevant ovarian (A) and head and neck (B) lesions are shown. (C) An ovarian carcinoma with clusters of tumour cells stained with Stalk-1. (D) Secreted APRIL (Aprily-2 staining) binds to syndecan-4 expressed by tumour cells in an ovarian carcinoma patient. Original magnifications were 40×.

calculated using the Kaplan–Meier method and compared with a log-rank test. All analyses were performed with the Prism software.

3. Results and discussion

We performed immunohistochemistry study for APRIL on tumour micro-arrays from patients with bladder ($n = 67$), ovarian ($n = 211$) and head and neck ($n = 128$) carcinomas with a pair of APRIL-specific antibodies selectively identifying cells producing APRIL (Stalk-1 reactivity) and secreted APRIL (Aprily-2 reactivity).[4] We observed 52%, 51% and 53% of APRIL^{high} lesions in bladder, ovary and head and neck carcinomas, respectively, with the Stalk-1 antibody. With the Aprily-2 antibody, results were very similar with 50%, 52% and 55% of APRIL^{high} lesions in bladder, ovary and head and neck carcinomas, respectively. Fig. 1A and B shows a relevant example of APRIL$^{low/neg}$ and APRIL^{high} lesions from ovarian and head and neck carcinomas. We previously reported relevant stainings for bladder carcinoma.[12] Tumour-infiltrating neutrophils (CD15$^+$ and elastase$^+$) were the major source of APRIL in lesions, consistent with the previous reports in solid tumours[12] and lymphomas.[4] In addition, 30%, 13% and 36% of lesions also contained tumour cells producing APRIL themselves in bladder, ovarian and head and neck carcinomas, respectively. APRIL production by these tumour cells was not homogeneous, but clustered (Fig. 1C), showing that carcinoma cells do not produce APRIL constitutively. In some lesions, Aprily-2 staining was very intense around carcinoma cells. Such concentration of secreted APRIL onto tumour cells was explained by APRIL binding to tumour HSPG, as illustrated in Fig. 1D with the colocalisation of secreted APRIL and the HSPG syndecan-4 expressed by an ovarian carcinoma tumour.

Clinically, APRIL upregulation was not specific to any carcinoma grade or stage (data not shown). We also analysed the 5-year DFS and OS by stratifying patients according to *in situ* APRIL level of expression. We observed that level of APRIL expression defined by Stalk-1 staining did not modulate DFS for bladder, ovarian and head and neck carcinomas (Fig. 2A). We first used a cut-off that was proven efficient to stratify diffuse large B-cell lymphoma patients (Ref. [4] and manuscript in preparation). Variations in the cut-off, or lower or higher, did not allow us to better observe DFS modulation. We also observed an absence of significant modulation when OS was studied, as well as when we monitored APRIL expression by Aprily-2 staining (data not shown). The tumour-promoting role of APRIL was originally observed in an experimental system with an autocrine production of APRIL by tumour cells.[5] Hence, we compared tumours with autocrine versus paracrine production (data not shown). Autocrine production of APRIL by tumour cells with any cut-off applied neither modulated DFS, when compared to paracrine production (Fig. 2B) nor did it modulate DFS when compared to low paracrine production of APRIL (data not shown). We also observed an absence of modulation for autocrine production of APRIL when analysing OS for these carcinomas (data not shown).

In conclusion, it is now widely accepted that host inflammatory reactions constitute an important part of a tumour development.[13] We did not find in this carcinoma study any significant correlation between expression level of the inflammatory APRIL and patient clinical outcome. Eventhough APRIL upregulation in carcinoma patients was in the range of the one observed in DLBCL, a lymphoma with a clinical development modulated by APRIL.[4] Taken together, this indicates that the proliferative/survival effects previously reported on solid tumours in pre-clinical assays, likely mediated by the signalling property of HSPG,[14] may not be strong enough to promote the development of solid tumours when they face chemotherapy in treated patients.

Conflict of interest statement

None declared.

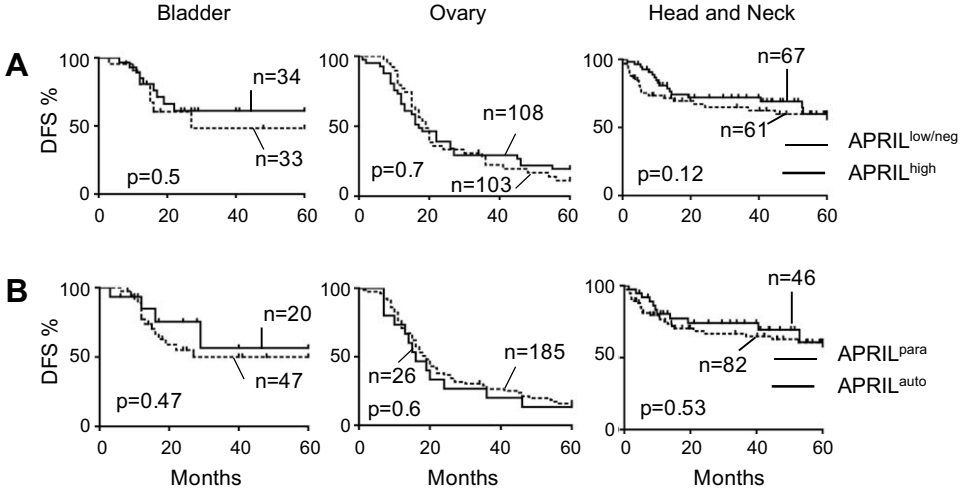

Fig. 2 – APRIL upregulation in carcinoma lesions does not modulate the clinical outcome of patients under treatment. Tumour lesions were stained with Stalk-1 to monitor APRIL level of expression. (A) DFS rates were calculated after patient stratification according to *in situ* APRIL level of expression. (B) DFS rates were compared between autocrine and paracrine level of APRIL expression.

Acknowledgements

This work was supported by the Docteur Henri Dubois Ferrière/Dinu Lipatti Foundation, the Leenaards foundation and the Swiss National Science Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.07.007.

REFERENCES

1. Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. *Annu Rev Immunol* 2003;**21**:231–64.
2. Jelinek DF, Darce JR. Human B lymphocyte malignancies: exploitation of BLyS and APRIL and their receptors. *Curr Dir Autoimmun* 2005;**8**:266–88.
3. Planelles L, Castillo-Gutierrez S, Medema JP, et al. APRIL but not BLyS serum levels are increased in chronic lymphocytic leukemia: prognostic relevance of APRIL for survival. *Haematologica* 2007;**92**(9):1284–5.
4. Schwaller J, Schneider P, Mhawech-Fauceglia P, et al. Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlates with human B-cell lymphoma aggressiveness. *Blood* 2007;**109**(1):331–8.
5. Hahne M, Kataoka T, Schroter M, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. *J Exp Med* 1998;**188**(6):1185–90.
6. Rennert P, Schneider P, Cachero TG, et al. A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. *J Exp Med* 2000;**192**(11):1677–84.
7. Roth W, Wagenknecht B, Klumpp A, et al. APRIL, a new member of the tumor necrosis factor family, modulates death ligand-induced apoptosis. *Cell Death Differ* 2001;**8**(4):403–10.
8. Darce JR, Arendt BK, Wu X, Jelinek DF. Regulated expression of BAFF-binding receptors during human B cell differentiation. *J Immunol* 2007;**179**(11):7276–86.
9. Ingold K, Zumsteg A, Tardivel A, et al. Identification of proteoglycans as the APRIL-specific binding partners. *J Exp Med* 2005;**201**(9):1375–83.
10. Hendriks J, Planelles L, de Jong-Odding J, et al. Heparan sulfate proteoglycan binding promotes APRIL-induced tumor cell proliferation. *Cell Death Differ* 2005;**12**(6):637–48.
11. Sasisekharan R, Shriver Z, Venkataraman G, Narayanasami U. Roles of heparan-sulphate glycosaminoglycans in cancer. *Nat Rev Cancer* 2002;**2**(7):521–8.
12. Mhawech-Fauceglia P, Kaya G, Sauter G, et al. The source of APRIL up-regulation in human solid tumor lesions. *J Leukocyte Biol* 2006;**80**(4):697–704.
13. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;**420**(6917):860–7.
14. Beauvais DM, Rapraeger AC. Syndecans in tumor cell adhesion and signaling. *Reprod Biol Endocrinol* 2004;**2**:3.