Table 1. A variable degree of cytoplasmic staining of tumor cells was observed and 37.3% (19/51 cases) of the analyzed tumors were evaluated to be positive, following the criteria described in Materials and methods.

Relationship between clinicopathologic findings and ATP7B expression

Table 1 summarizes the relationship between clinicopathologic features and ATP7B expression in endometrial carcinomas. No significant association was found between ATP7B expression and age, FIGO stage, or histopathologic subtypes (Table 1). However, ATP7B positivity was correlated with tumor grades, i.e., ATP7B positivities in the degree of G2 and G3 carcinoma were significantly higher than that in G1 carcinoma with chi-square test ($P = 0.019$).

Prognostic relevance of ATP7B expression

Kaplan–Meier estimates of disease-free or overall survival were plotted in Figs. 2A and B. The patients with ATP7B-positive carcinomas had poorer disease-free survival and overall survival than those with ATP7B-negative tumors with log-rank test ($P < 0.01$). We performed analysis using Cox's proportional hazards model with the adjustment of age, stage, and grade. In univariate analysis, relative risks of overall survival were 1.06 (0.98–1.16 $P = 0.17$) with age, 8.13 (2.11–31.3, $P < 0.01$) with grade, 2.78 (1.18–6.54, $P = 0.02$) with stage, and 14.31 (1.56–130.9, $P = 0.019$) with ATP7B expression. With multivariate analysis, we found significant relation between overall survival and grade: RR 10.03 (1.22–82.2, $P = 0.032$). With regard to ATP7B expression, marginal relation between overall survival and ATP7B expression was found but it was not statistically significant; RR 47.1 (0.69–320.8, $P = 0.074$).

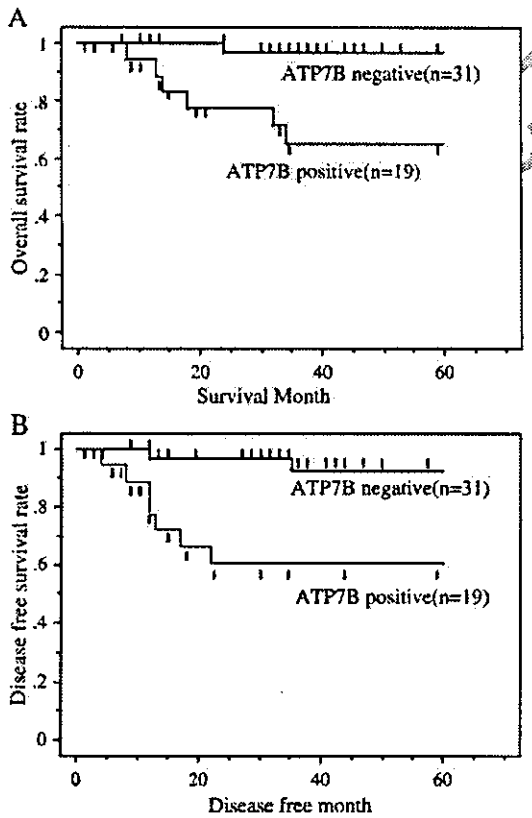

Fig. 2. Kaplan–Meier survival curves of patients with endometrial carcinoma. (A) Overall survival curve. (B) Disease-free survival curve. Comparison of survival curve for patients whose tumors were positive for ATP7B with that negative for ATP7B expression. The differences were analyzed with log-rank test.

Discussion

Several important findings are presented in this report. First of all, ATP7B was expressed in human endometrial carcinoma as assessed by immunohistochemistry (Fig. 1). ATP7B immunoreactivity in tissues was detected as granular cytoplasmic staining. In agreement with this observation, ATP7B has been reported to be abundant in the Golgi apparatus [18]. These findings are the first evidence(s) that ATP7B is expressed in endometrial carcinoma. Secondly, ATP7B expression in the degree of differentiation of G2 and G3 endometrial carcinoma was more frequent than that of G1 carcinoma. Thirdly, no expression of ATP7B gene and protein could be detected in adjacent non-neoplastic tissues. The fact that the expression level of ATP7B was not detected in normal endometrial tissues raised the possibility that ATP7B might be involved in transformation of a normal cell to a malignant tumor cell and/or differentiation of carcinoma cell.

ATP7B expression assessed by immunohistochemistry has the potential to become a prognostic factor for survival in patients with endometrial carcinoma treated with cisplatin-based chemotherapy. In univariate analysis, ATP7B expression was significant relation with survival. However, this relation was marginal in multivariate analysis. This seemed to be due to the strong correlation between ATP7B and grade, which is of special clinical interest because undifferentiated carcinomas is usually more refractory to therapy. The same relationship with respect to ATP7B and endometrial cancer has also been found for ovarian carcinoma [19,22]. A priori knowledge of the ATP7B expression may be important in the choice of therapy. It will be necessary to study comparison of clinical response to cisplatin-based chemotherapy with ATP7B expression in endometrial carcinoma. In the future, drugs targeting ATP7B may be useful in combination with cisplatin-based chemotherapy for the improvement of survival rate of gynecologic cancers.

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References

[1] Wingo PA, Tong T, Bolden S. Cancer statistics, 1995. CA Cancer J Clin 1995;45:8–30.
[2] Riordan JR, Deuchars K, Kartner N, Alon N, Trent J, Ling V. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature 1985;316:817–9.
[3] Scheffer GL, Wijngaard PL, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, et al. The drug resistance-related protein LRP is the human major vault protein. Nat Med 1995;1:578–82.
[4] Ueda K, Cardarelli C, Gottesman MM, Pastan I. Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci U S A 1987;84:3004–8.
[5] Cole SP, Sparks KE, Fraser K, Loe DW, Grant CE, Wilson GM, et al. Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. Cancer Res 1994;54:5902–10.
[6] Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, et al. Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 1989;81:116–24.
[7] Nooter K, Bosman FT, Burger H, van Wingerden KE, Flens MJ, Scheper RJ, et al. Expression of the multidrug resistance-associated protein (MRP) gene in primary non-small-cell lung cancer. Ann Oncol 1996;7:75–81.
[8] Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59:8–13.
[9] Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells [published erratum appears in Proc Natl Acad Sci U S A 1999 Mar 2;96(5):2569]. Proc Natl Acad Sci U S A 1998;95:15665–70.
[10] Allen JD, Brinkhuis RF, Wijnholds J, Schinkel AH. The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. Cancer Res 1999;59:4237–41.
[11] Volk EL, Rohde K, Rhee M, McGuire JJ, Doyle LA, Ross DD, et al. Methotrexate cross-resistance in a mitoxantrone-selected multidrug-resistant MCF7 breast cancer cell line is attributable to enhanced energy-dependent drug efflux [in process citation]. Cancer Res 2000;60:3514–21.
[12] Komatsu M, Sumizawa T, Mutoh M, Chen ZS, Terada K, Furukawa T, et al. Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. Cancer Res 2000;60:1312–6.
[13] Harris ED, Quian Y, Tiffany-Castiglioni E, Lacy AR, Reddy MC. Functional analysis of copper homeostasis in cell culture models: a new perspective on internal copper transport. Am J Clin Nutr 1998;67 (Suppl.): 988S–95S.
[14] Roberts EA, Cox DW. Wilson disease. Bailliere's Clin Gastroenterol 1998;12:237–56.
[15] Terada K, Schilsky ML, Miura N, Sugiyama T. ATP7B (WND) protein. Int J Biochem Cell Biol 1998;30:1063–7.
[16] Solioz M, Vulpe C. CPx-type ATPases: a class of P-type ATPases that pump heavy metals. Trends Biochem Sci 1996;21:237–41.
[17] Gupta A, Matsui K, Lo JF, Silver S. Molecular basis for resistance to silver cations in Salmonella. Nat Med 1999;5:183–8.
[18] Petrukhin K, Lutsenko S, Chernov I, Ross BM, Kaplan JH, Gilliam TC. Characterization of the Wilson disease gene encoding a P-type copper transporting ATPase: genomic organization, alternative splicing, and structure/function predictions. Hum Mol Genet 1994;3: 1647–56.
[19] Nakayama K, Kanzaki A, Ogawa K, Miyazaki K, Neamati N, Takebayashi Y. COPPER-transporting P-type ATPase (ATP7B) as a cisplatin-based chemoresistance marker in ovarian carcinoma: comparative analysis with expression of MDR1, MRP1, MRP2, LRP and BCRP. Int J Cancer 2002;101:488–95.
[20] Yang XL, Miura N, Kawarada Y, Terada K, Petrukhin K, Gilliam T, et al. Two forms of Wilson disease protein produced by alternative splicing are localized in distinct cellular compartments. Biochem J 1997;326:897–902.
[21] Chan HS, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, et al. P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 1991;325:1608–14.
[22] Nakayama K, Kanzaki A, Terada K, et al. Prognostic value of the Cu-transporting ATPase in ovarian carcinoma patients receiving cisplatin-based chemotherapy. Clin Cancer Res 2004;10:2804–11.

Precis
This study investigated the role of ATP7B expression in endometrial carcinoma using 51 patients.