Enhanced casein kinase II activity in human tumour cell cultures

Katja Prowald, Hans Fischer* and Olaf-Georg Issinger+

Institut für Humangenetik, Universität des Saarlandes, D-6650 Homburg-3, and **Institut für Virusforschung, Deutsches Krebsforschungszentrum D-6900 Heidelberg, FRG*

Received 23 August 1984

Casein kinase II (CKII) activity is enhanced as much as 2–3 fold in established and 4–5-fold in transformed human cell lines when compared to that of fibroblasts and primary human tumour cell cultures where CKII activity never exceeded a basic level. The high activity of CKII in transformed cells and in established cell lines was reduced to about the same basic level after treatment with heparin, a highly specific inhibitor of CKII activity. The activity of the cAMP-dependent protein kinase was virtually the same in fibroblasts and various human tumour cell lines investigated.

Casein kinase *Human* *Tumor cell culture* *Phosphorylation* *Transformation*

1. INTRODUCTION

When a cancer is formed or a cell line established in vitro, the barrier of programmed senescence of cells must be overcome. A varied body of evidence indicates that protein kinases are involved in the path towards tumorigenicity [1–6].

Recent reports on elevated cAMP-independent protein kinase activity in leukaemic cells and solid glioblastoma compared to the corresponding controls, i.e., lymphocytes and brain tissue [7–9], prompted us to search for an increased cAMP-independent protein kinase activity in various tumour cell cultures and fibroblasts derived from the same patients. We focussed our attention on casein kinase II (CKII) since this enzyme has been shown to be involved in the phosphorylation of cellular compounds such as translational factors [10,11], glycogen synthetase [12–14] and the regulatory subunit of the R_2C_2 protein kinase [15] in vivo and in vitro. Furthermore, CKII can be unambigously characterized even in crude cell lysates by its unique biochemical and biophysical properties, i.e., (i) its elution profile on DEAE and phosphocellulose, (ii) its ability to utilize ATP and GTP in the phosphotransferase reaction, (iii) its sensitivity to heparin [16,17] and (iv) its substrate specificity for certain polypeptides of protein synthesis factors eIF-2 and eIF-3 [10,11,17]. Here we present evidence that CKII activity is enhanced in established and transformed human tumour cells when compared to that of fibroblasts and primary human tumour cell cultures.

2. MATERIALS AND METHODS

2.1. *Biological material and cell culture*

Primary cell cultures (T2518, T2496, meningiomas; T2565, glioblastoma; T2554, brain metastasis of an anaplastic carcinoma) were derived from biopsies immediately after removal from the patient and studied in early subcultures. Fibrosarcoma cell line HT-1080 is commercially available (Flow Lab.). The established and experimentally transformed glioblastoma cell lines (T406, T508, HeRo) were derived from tumour biopsies and studied after 60 subcultures. Glioblastoma cell lines HeRo and HeRo-SV are

+ To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

described in detail elsewhere [18]. HeLa cells were from Dr K. Köhler, University of Stuttgart.

Cells were grown in 25-cm^2 Falcon flasks to a cell density of 1–5 × 10^5 cells in M199 medium supplemented with 10% newborn calf serum (NCS) or in MEM supplemented with 10% fetal calf serum (FCS). Details are given in the corresponding figure legends.

2.2. *Determination of casein kinase II activity*

Cells were isolated by trypsination, washed twice in phosphate-buffered saline (PBS) and were then suspended in extraction buffer (20 mM Tris-Cl^- (pH 7.2), 100 mM NaCl, 100 μM EDTA, 7 mM 2-mercaptoethanol, 10 μM PMSF). Cells were homogenized by applying 20 strokes with a tight fitting Dounce homogenizer. Nuclei were removed by centrifugation at 5000 × *g* for 10 min at 4°C. The postnuclear supernatant was used as an enzyme source. The protein concentration of the cytoplasmic extracts was adjusted to 1 mg · ml^{-1}. 5-μl aliquots were used for the enzyme reaction.

The enzyme mixture contained: 100 mM Mes-OH^- (pH 6.9), 130 mM NaCl, 5 mM $MgCl_2$, 0.5% casein (dephosphorylated), 10 mM DTT, 100 μM ATP; the specific activity of the [γ-^{32}P]ATP was 500 cpm · $pmol^{-1}$. Total assay volume was 45 μl. Incubation was at 37°C for 15 min. 30-μl aliquots were removed, spotted onto filter squares and precipitated with trichloroacetic acid. Filters were counted in a scintillation cocktail. 1 enzyme unit is defined as the transfer of 1 pmol phosphorus · min^{-1} · mg^{-1} protein at 37°C. cAMP-dependent protein kinase activity was determined as in [19].

2.3. *Immunofluorescent staining*

Selected tissue cultures (meningioma, T2496; glioblastoma HeRo, transformed glioblastoma HeRo-SV) were grown on small slides in Falcon flasks. For staining, the slides were removed and washed 3 times in PBS. The cells were fixed in acetone at 4°C for 15 min. Slides were washed again twice in PBS and then a 0.2 ml IgG fraction from PAb (clone 412) mouse mouse hybridoma which specifically recognizes the large T-antigen was added. This was followed by an incubation at 37°C for 30 min. After washing the cells 3 times with PBS, 0.2 ml FITC-conjugated goat F(ab')$_2$ anti-mouse IgG-FITC (TAG), 1:50 diluted, was added. Staining was carried out at 37°C for 60 min. To remove the fluoresceinated material, cells were washed again in PBS and finally embedded in PBS-glycerol.

3. RESULTS AND DISCUSSION

Primary cell cultures from benign or malign tumours degenerate generally after 10–15 passages; the reason for this phenomenon is still unknown. However, in a few cases primary cell lines become immortalized spontaneously and exhibit the typical features of an established cell line (e.g., higher proliferation rates and unlimited growth) [20].

Fig.1 shows growth curves of such a spontaneously immortalized glioblastoma cell line, before and after transformation with SV40. We see that the SV40 transformed cells exhibit an approx. 30% higher growth rate than the untransformed,

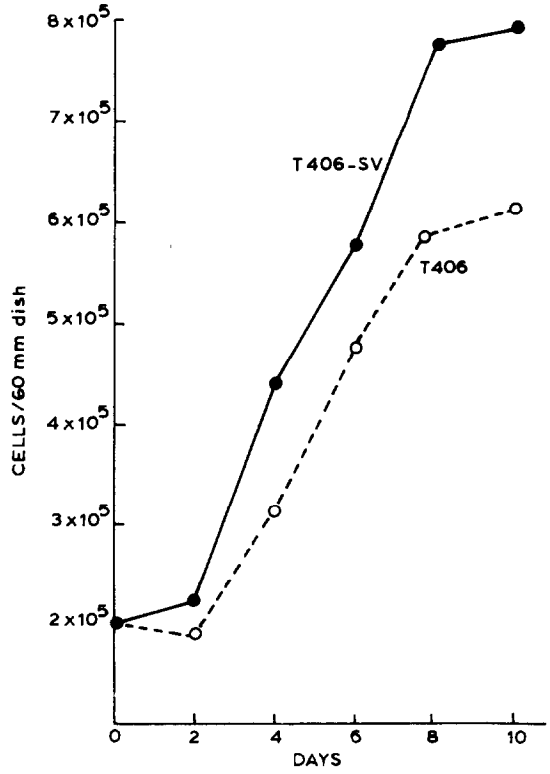

Fig.1. Growth curves of glioblastoma (T406) and transformed glioblastoma cells (T406-SV) under optimal conditions in MEM and in the presence of 10% fetal calf serum.

yet established counterparts. Furthermore, the transformed cell strains were free of infectious virus, grew to higher saturation densities and grew in soft agar. We investigated the CKII activity in all 3 types of cell culture, i.e., primary cell cultures, established and transformed cell lines, using a variety of different human tumour cell cultures of different origin.

Fig.2A shows the CKII activity in primary tissue cultures from human tumour cells and fibroblasts from the same patients. The enzyme activity varied between 200 and 500 $U \cdot mg^{-1}$ protein. Maximum observed differences between fibroblasts and primary human tumour cells from the same patient ranged from 15 to 30%. In contrast to the low CKII activities detected in primary tumour cell cultures the observed CKII activities were 700–1000 $U \cdot mg^{-1}$ protein in established and 1000–1600 $U \cdot mg^{-1}$ protein in transformed cell lines (fig.2B,C). With the exception of HeLa, all cells were transformed with SV40 (fig.2C). These data match well with reports on a cAMP-independent protein kinase activity in human leukaemic cells and solid glioblastomas which was usually twice that of the corresponding controls, i.e., lymphocytes and brain tissue [7–9]. The high

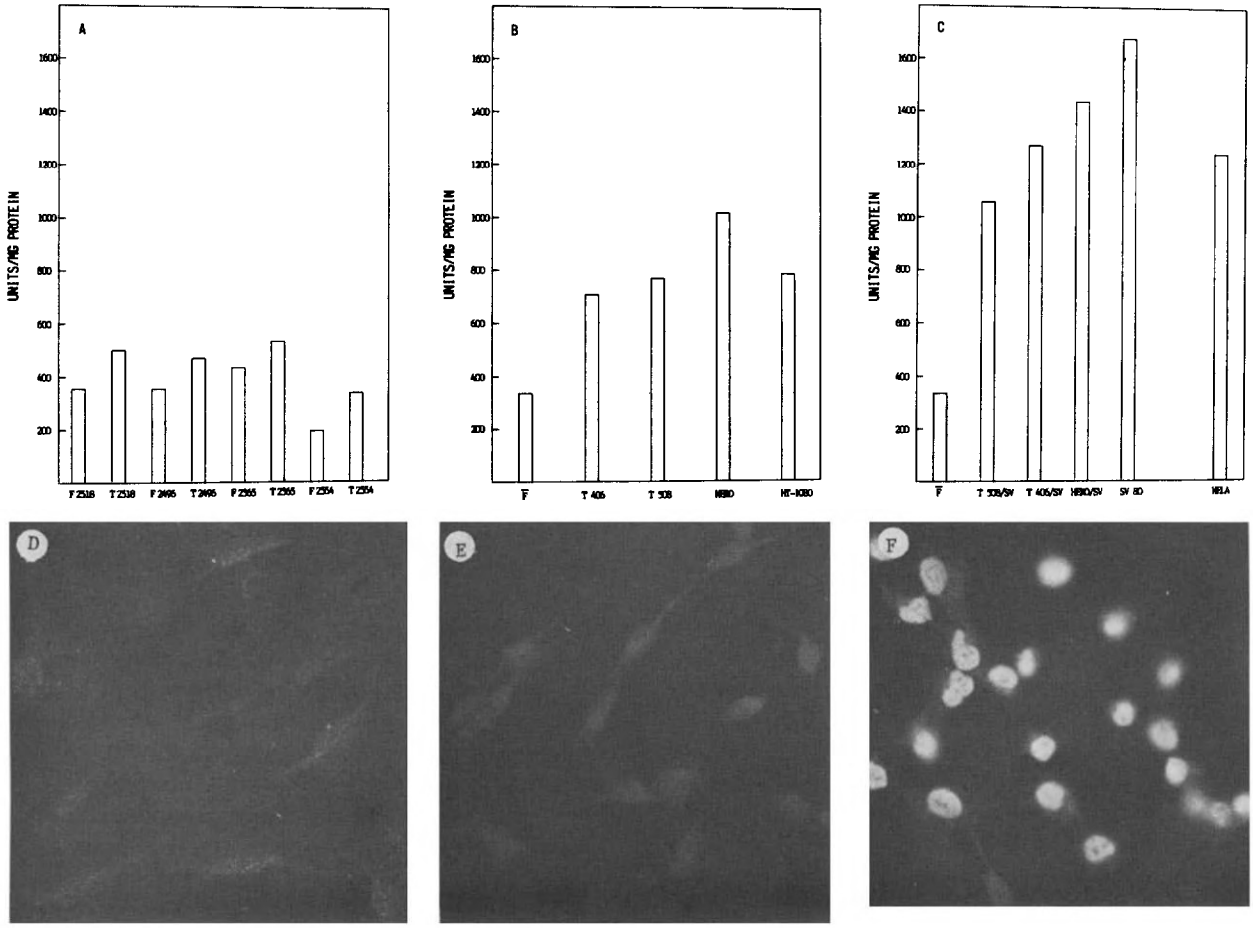

Fig.2. Casein kinase II activity in cytoplasmic extracts from (A) fibroblasts and human primary tumour cell cultures; (B) established cell lines and (C) transformed cell lines. Enzyme activity is expressed in units $\cdot mg^{-1}$ protein at 37°C: Tumour (T) fibroblast (F). Classification of the tumours is given in section 2. $\bar{F}$ indicates average enzyme activity from all fibroblasts investigated. Immunofluorescent staining of the large T-antigen in primary cell cultures [meningioma, T2496 (D)], established [glioblastoma, HeRo (E)] and transformed cells [glioblastoma, HeRo-SV (F)].

activity of CKII in transformed cells compared to established cell lines was reduced to about the same basic level after heparin treatment and also irrespective of the kind of phorphoryl donor used in the enzyme assay (fig.3A,B). If we were indeed measuring different kinds of protein kinase activities in established and transformed cell lines we would not expect this result. Hence, we consider these observations as strong evidence for a concept of gradual activation of CKII activity in the different tissue cultures investigated which can be shut off by a highly specific inhibitor.

CKII activity was enhanced in established and transformed cells; cAMP-dependent protein kinase activity was about 150 $U \cdot mg^{-1}$ protein in all cell lines investigated so far.

Fig.2D–F shows representative immunofluorescent micrographs of cell lines which were analyzed for the presence of large T-antigen. Only virus-transformed cells (e.g., HeRo-SV, T408-SV, T506-SV and SV80) gave a positive nuclear fluorescence as shown in fig.2F. As HeLa cells do not contain large T-antigen but still show CKII activity well in the range of SV40 transformed cells, we tend to believe that enhanced CKII activity reflects a secondary effect which is possibly caused by the establishment and transformation of the cells by another exogenous agent. This may be directly related to higher transcriptional and translational rates commonly found in tumour cells than, for instance, to the presence of large T-antigen [21–26]. The increase in CKII activity seems acceptable in view of the observed stimulation of cellular protein phosphorylation shown in transformed cells [27–30] especially as CKII has been shown to phosphorylate stoichiometrically initiation factors of protein synthesis [10,11]. Furthermore, it was shown that glycerate-2,3-P_2, which is involved in the regulation of the glycolytic flux by modulating the activity of hexokinase and phosphofructokinase, inhibits the phosphorylation of the β-subunit of initiation factor 2 (eIF-2) by CKII [31].

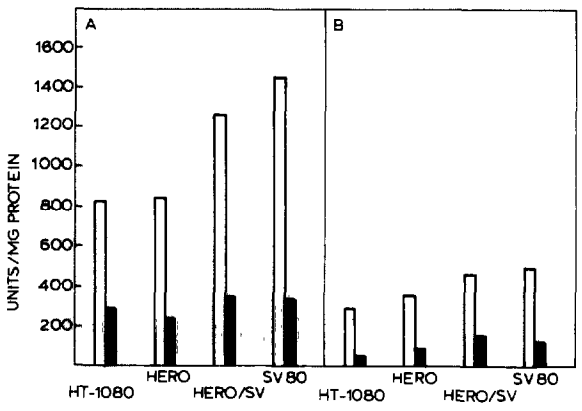

Fig.3. Inhibition of CKII activity by heparin. CKII activity was tested in the presence of either ATP (A) or GTP (B). Solid columns show the CKII activity in the presence of 1 $\mu g \cdot ml^{-1}$ heparin.

The relationship between the initiation of translation and glycolysis is intriguing, especially if one considers other observations where it was shown that most of the tumour cells are capable of extensive anaerobic glycolysis [32]. These observations may turn out to be more apparent than real, as we still do not know whether CKII plays a specific or unspecific role in carcinogenesis and as other protein kinases are also present in tumour cells [1–6].

Although we do not yet understand the relationship between establishment function, transformation and enhanced CKII activity, the observed differences in the activity of this particular enzyme in the human cell lines investigated make it an ideal marker to distinguish between primary cell cultures with a limited life-span and both established and transformed cell lines which have undergone immortalization.

ACKNOWLEDGMENTS

We thank Ms Monika Thönnes for assistance in tissue culture, and Ms Angela Rance, Dr Hildburg Beier and Professor K.D. Zang for critically reading the manuscript. This work was funded by DFG grant Is19/9-1.

REFERENCES

[1] Collet, M.S., Brugge, J.S. and Erikson, R.L. (1978) Cell 15, 1363–1369.
[2] Hunter, T. and Cooper, J.A. (1981) Cell 24, 741–752.
[3] Cooper, J.A., Bowen-Pope, D.F., Raines, E. and Hunter, T. (1982) Cell 31, 263–273.
[4] Petruzelli, L.M., Sabayashi, G., Smith, C.J., Cobb, M.H., Rubin, C.S. and Rosen, O. (1982) Proc. Natl. Acad. Sci. USA 79, 6792–6796.

[5] Gilmore, T. and Martin, G.S. (1983) Nature 306, 487–490.
[6] Land, H., Parada, L.F. and Weinberg, R.A. (1983) Science 222, 771–777.
[7] Elias, L., Li, A.P. and Longmire, J. (1981) Cancer Res. 43, 2182–2188.
[8] Frattola, L., Canal, N., Ferrarese, C., Tonini, C., Tonon, G., Villani, R. and Trabucchi, M. (1983) Cancer Res. 43, 1321–1324.
[9] Pena, J.M., Itarte, E., Domingo, A. and Cusso, R. (1983) Cancer Res. 43, 1172–1175.
[10] Issinger, O.-G., Benne, R., Hershey, J.W.B. and Traut, R.R. (1976) J. Biol. Chem. 251, 6471–6474.
[11] Traugh, J.A., Tahara, S.M., Sharp, S.B., Safer, B. and Merrick, W.C. (1976) Nature 263, 163–165.
[12] Nimmo, H.G. and Cohen, P. (1974) FEBS Lett. 47, 162–166.
[13] Huang, K., Itarte, E., Singh, T.J. and Akatsuka, A. (1982) J. Biol. Chem. 257, 3236–3242.
[14] Picton, C., Woodgett, J., Hemmings, B. and Cohen, P. (1982) FEBS Lett. 150, 191–196.
[15] Carmichael, D.E., Geahlen, R.L., Allen, S.M. and Krebs, E.G. (1982) J. Biol. Chem. 257, 10440–10445.
[16] Feige, J.J., Pirollet, F., Cochet, C. and Chambaz, E.M. (1980) FEBS Lett. 121, 139–142.
[17] Hathaway, G.M. and Traugh, J.A. (1982) Curr. Top. Cell. Regul. 21, 101–127.
[18] Fischer, H., Schwechheimer, K., Heider, M., Bernhardt, S. and Zang, K.D. (1984) Cancer Genet. Cytogenet., in press.
[19] Issinger, O.-G., Beier, H., Speichermann, N., Flokerzi, V. and Hofmann, F. (1980) Biochem. J. 185, 89–99.
[20] Ponten, J. (1971) Biochim. Biophys. Acta 458, 397–422.
[21] Pardee, A.B., Campisi, J. and Croy, R.G. (1982) Ann. NY Acad. Sci. 397, 121–129.
[22] Whelley, S., Ide, T. and Baserga, R. (1978) Virology 88, 82–91.
[23] Ide, T., Whelley, S. and Baserga, R. (1977) Proc. Natl. Acad. Sci. USA 74, 3189–3192.
[24] Postel, E.H. and Levine, A.J. (1976) Virology 73, 206–215.
[25] Chou, J.V. and Martin, R.G. (1975) J. Virol. 15, 146–160.
[26] Tijan, R., Fey, G. and Graessmann, A. (1978) Proc. Natl. Acad. Sci. USA 75, 1279–1283.
[27] Pumo, D.E., Stein, G.S. and Kleinsmith, L.J. (1975) Biochim. Biophys. Acta 402, 125–130.
[28] Segawa, K., Yamaguchi, N. and Oda, K. (1977) J. Virol. 22, 679–693.
[29] Segawa, T., Oda, K., Yuasa, Y., Shiroki, K. and Shimojo, H. (1978) J. Virol. 27, 800–808.
[30] Epstein, J., Breslow, L. and Fontaine, J.J. (1979) Proc. Natl. Acad. Sci. USA 76, 6396–6400.
[31] Gonzatti-Haces, M.I. and Traugh, J.A. (1982) J. Biol. Chem. 257, 6642–6645.
[32] Warburg, O. and Christian, F. (1942) Naturwissenschaften Heft 48/49, 731.