EUROPEAN JOURNAL OF CANCER 43 (2007) 1483–1492

available at www.sciencedirect.com

journal homepage: www.ejconline.com

Proteomic co-expression of cyclin-dependent kinases 1 and 4 in human cancer cells

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ARTICLE INFO

Article history:
Received 4 January 2007
Received in revised form 8 March 2007
Accepted 15 March 2007
Available online 3 May 2007

Keywords:
Cyclin-dependent kinase
Cdk1
Cdk4
Western blotting
Cell lines
Human
Cancer

ABSTRACT

The roles of the cyclin-dependent kinases Cdk2, Cdk4 and Cdk6 and their complementary cyclin partners in moving cells from a quiescent state into active DNA synthesis are presently undergoing re-evaluation. Normal cell cycling now appears possible in the absence of any of these molecular controlling factors whilst certain cell-cycle control kinases, such as Cdk4, appear to be mandatory for cancer cell growth. Here, we describe a unique relationship between proteomic expression of Cdk1 and Cdk4 in human cancer cell lines and data from clinical malignant melanoma. The relationship was not present in normal diploid keratinocytes and fibroblasts. We suggest that the much tighter spread of Cdk1/Cdk4 ratios in human cancer cells compared to normal cells may selectively benefit the cancer cell and thus provide a potential novel anticancer target.

1. Introduction

The complex series of events by which mammalian cells replicate is classically controlled by the sequential expression of cyclins, which with their cyclin-dependent kinase partners (Cdks) propel cells through the successive G1, S, G2 and Mitotic phases of the cell-cycle.[1–3] The activity of cyclin/Cdk holoenzymes has been shown to be regulated both by upstream positive signalling pathways[4] and by negative signalling molecules such as p53 and cyclin-dependent kinase inhibitors (CDKIs).[5] The downstream targets of Cdks activated by their cyclin co-partners are the retinoblastoma protein (pRb) and associated pRb family members.[6,7]

Unsurprisingly, the change from normal cells to cancer cells is frequently accompanied by alterations in the proteomic expression of cyclins, Cdks, CDKIs and their associated upstream regulatory molecules and downstream effector molecules.[8–10]

Recently selective disruption of the genes responsible for the expression of D- and E-type cyclins and their cyclin-dependent kinase partners, Cdk2, Cdk4 and Cdk6, indicates that none of these proteins are indispensable for normal cell division and normal foetal development can occur in their absence.[11] Transformed cells, however, do not appear to share the 'indifference' to the loss of various cyclins and Cdks shown by normal developing cells, but can be profoundly sensitive to the loss of D- and E-type cyclins and their Cdk4 and Cdk6, but not Cdk2, partners.[12] In particular, carcinogenesis in mouse models does not occur in the absence of Cdk4.[13,14]

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.03.014

Cyclin D1/Cdk4 complexes are now known to exhibit a broader set of functions than was apparent a decade ago. The kinase activity of cyclin D1/Cdk4 is not limited to phosphorylation of the Rb family as substrates, but also extends to phosphorylation of Smad3, which itself influences TGF-β growth inhibition.[15] Also cyclinD1/Cdk4 complexes are able to function through a non-kinase mechanism by which they sequester p27KIP1 and p21WAF1/Cip1, thus releasing downstream cyclinE/Cdk2 from the inhibitory effects of these cyclin-dependent kinase inhibitors.[11,16] Moreover, D-type cyclins can act on their own, independently of Cdk binding, when they activate or repress transcription factors.[17,18]

Here, we report that proteomic co-expression to a high degree of significance of Cdk1 and Cdk4 occurs across a wide range of human *in vitro* cancer cell lines, but not in short term cultured normal diploid keratinocytes or fibroblasts. Similar proteomic co-expression of Cdk1 and Cdk4 can also be found on re-analysis of protein expression from clinical malignant melanoma previously reported in the literature.[19] Given the potential therapeutic importance of Cdk1 and Cdk4 inhibition, this paper explores the Cdk1/Cdk4 co-relationship we have identified and proposes that it may provide a potential target for rational anticancer drug development.

2. Materials and methods

2.1. Cell lines and culture conditions

The 20 human cell lines deployed[20–22] are listed, with their histological classification and origins in Table 1. All are well established. Sufficient stocks were prepared for 24 month experimentation to minimise the influence of genetic drift. Cell lines were routinely maintained in DMEM, except RT112 and H322 which grew most successfully in RPMI and MGHU-1 which are grown in Ham's F12 medium. All media were supplemented with 10% heat-inactivated foetal calf serum (HIFCS), glutamine and penicillin/streptomycin.

2.2. Cell lysates for Western blotting

10^7 Cells growing asynchronously in 162 cm^2 tissue culture flasks (Costar Ltd., High Wycombe, Bucks) until they were pre-confluent and still growing exponentially were removed by trypsinisation, washed and resuspended at 3×10^7 cells per ml of lysis buffer (×1 stock solution: 10% (w/v) SDS 10 ml, 0.5 M Tris, pH 6.8, glycerol 10 ml, double distilled water 62. To 10 ml of stock solution was added 100 μl of 10 μM Leupeptin plus 10 μl of 100 μM PMSF). Protein estimations were performed using a bicinchoninic acid/BSA assay (Sigma), measured spectrophotometrically at 526 nm and the final concentration of the lysates was adjusted to 300 μg total cellular protein per 100 μl of lysis buffer.

2.3. Western blotting

The method of quantitative Western blotting used here has already been described in detail.[23] Briefly, two independent Western blottings with lysates for each cell line loaded in pairs on each gel were carried out using 150 μg of total cellular protein in 50 μl of lysate buffer. After electrophoresis at 16 °C using 60 V over 16 h and a constant current of 500 mA, the proteins were transferred to nitrocellulose at 22 °C over 16 h using a semi-dry blotting apparatus (Biorad, Richmond, CA). Primary antibodies were Cdk1 – mouse monoclonal sc-54 (1/250) (Santa Cruz Biotechnology); Cdk2 – (M2) rabbit polyclonal sc-163 (1/250) (Santa Cruz Biotechnology); Cdk4 – rabbit polyclonal sc-260 (1/250) (Santa Cruz Biotechnology); Cyclin A (BF683) – mouse monoclonal sc-239 (1/125) (Santa Cruz Biotechnology); Cyclin B1 – mouse monoclonal 14561C (1/1000) (Pharmingen); Cyclin D1 – mouse monoclonal 14841C (1/125) (Pharmingen); and Cyclin E – (HE12) mouse monoclonal sc-247 (1/125) (Santa Cruz Biotechnology). Quantitation of each protein was carried out by measurement of either optical density on a Shimadzu scanning densitometer with tungsten light and expressed as OD

Table 1 – Histological subtype and source of human *in-vitro* cancer cell lines

Cell line	Histology	Source
2780	Ovarian carcinoma	ECACC 93112519
A431	Skin squamous carcinoma	ECACC 85090402
A549	Lung adenocarcinoma	ECACC 86012804
AT5	Fibroblast ataxia telangetasia	IRC Sutton J Peacock
COR L23	Lung large cell carcinoma	ECACC 93051118
G361	Melanoma	ECACC 88030461
H322	Lung small cell carcinoma	ECACC 95111734
HELA	Cervix squamous carcinoma	ECACC 93021013
HEP2	Larynx squamous carcinoma	ECACC 86030501
HRT18	Rectum adenocarcinoma	ECACC 86040306
HT29/5	Colon adenocarcinoma clone 5	ECACC 91072201 Parent
HX142	Neuroblastoma	IRC Sutton J Peacock
HX34	Skin melanoma	IRC Sutton J Peacock
I407	Intestine embryonic	ATCC CCL6 via ICN
KB	Oral epidermoid carcinoma	ECACC 94050408
SK-MEL-3	Melanoma	ATCC HTB-69
MGHU-1	Bladder transitional cell carcinoma	IRC Sutton J Peacock
MOR	Lung adenocarcinoma	MRC CORU Cambridge
OAW42	Ovarian Carcinoma	ECACC 85073102
RT112	Bladder transitional cell carcinoma	ECACC 85061106

units per 150 μg of total cellular protein or by enhanced chemiluminescence (ECL). Band images produced by either modalities were read on 'Phoretix' analysis software and expressed as peak intensities per 150 μg of total cellular protein.

2.4. Normalising and ranking Western blot data for intercomparison of proteomic expression

Each Western blot was run with the full range of different cell lines. In order to compare the levels of different proteins between the cell lines whilst compensating for inter-gel variability and different intensities of antibody binding from epitope to epitope, the mean OD value for all the lines was calculated and the relative OD for protein in each individual cell line was normalised to the mean OD and multiplied by an arbitrary value of 5.0:

$$\frac{\text{OD of each individual lane } (x_1, x_2, x_3, x_4, \ldots, X_n) \times 5.0}{\text{mean OD } (\bar{x})}$$

This procedure allowed all the proteins tested to be ranked on the same scale, thus enabling valid intercomparisons of relative expression.

2.5. Measurement of Cdk1 and Cdk4 protein half-lives

Exponentially growing cells were cultured in T75 cm^3 culture flasks in their appropriate growth medium. After 24 h the medium was replaced with medium minus the amino acids such as methionine, cystine and glutamine and the cells were incubated at 37 °C for 1 h. One hundred and eighty five Megabequerels (MBqs) of ^{35}S Methionine was added to 20 ml of medium minus the amino acids such as methionine, cystine and glutamine. This medium volume was evenly divided between the flasks. Cells were returned to the

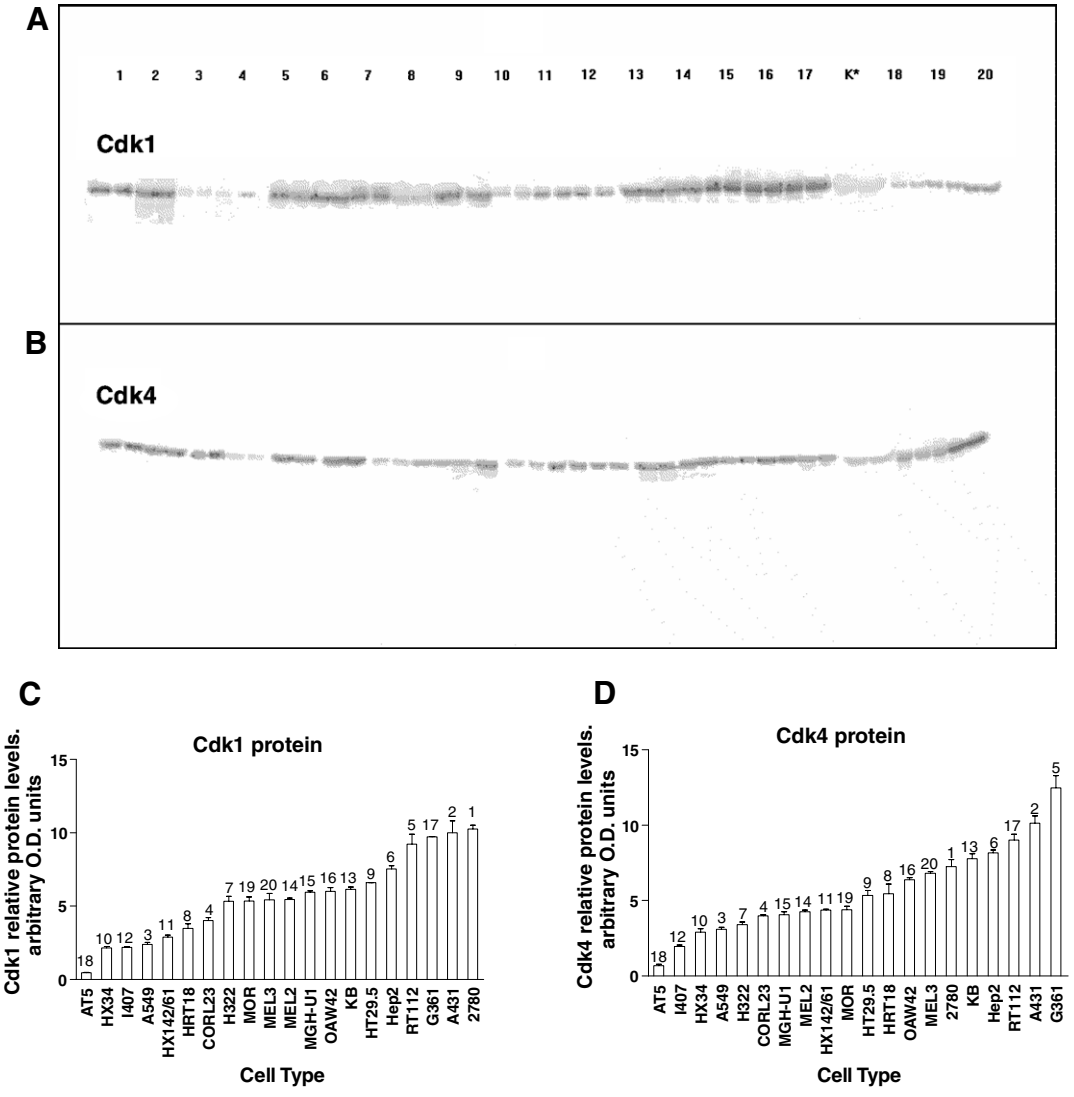

Fig. 1 – Western blots of human *in vitro* cell lines and short-term cultured keratinocytes. After exposure to primary antibodies anti-Cdk1 mouse monoclonal SC-54 (Panel A) and rabbit polyclonal anti-Cdk4 sc-260 (Panel B). Panels C and D show histograms ±1 SEM of ataxia telangectasia (AT5*), I407 and 18 human cancer *in vitro* cell lines in the same ranked order. Numbers above each histogram relate to the numbered lanes in panels A and B.

incubator with agitation at 37 °C for 4 h. Radiolabel was chased by a 37 °C PBS wash followed by a normal medium wash and the cells were incubated at 37 °C. At pre-designated time points cells were washed with 5 ml of ice-cold PBS and resuspended in 2 ml of ice-cold NP-40 lysis buffer. The cells were scraped from flasks and transferred into pre-cooled eppendorfs. The cell lysate slurry was passed several times through a 23 gauge needle and 20 μl aliquots were removed for protein estimation. The remainder of the cell lysate was stored at −80 °C.

Following immunoprecipitation, the radiolabelled samples were analysed by 12% PAGE, the gel was fixed (methanol:glacial acetic acid:water (20:10:70)) for 1 h at room temperature, soaked in an equilibrating hydration solution (methanol:glycerol:water (20:3:77)) on a rocking platform for 16 h, dried down at 65 °C for 3 h under vacuum and exposed to photographic film in the dark at −80 °C for 3 days. The film was scanned using a flatbed scanner at 800 dpi (Epson GT-8000), the images produced were captured to a gel analysis software package (Phoretix) and analysed by statistical software (Prism 2.01).

2.6. *CDK4 transfected RAMA 37 cells*

Eight positive clones containing the vehicle were isolated from RAMA 37 (rat mammary epithelial) cells[24] containing

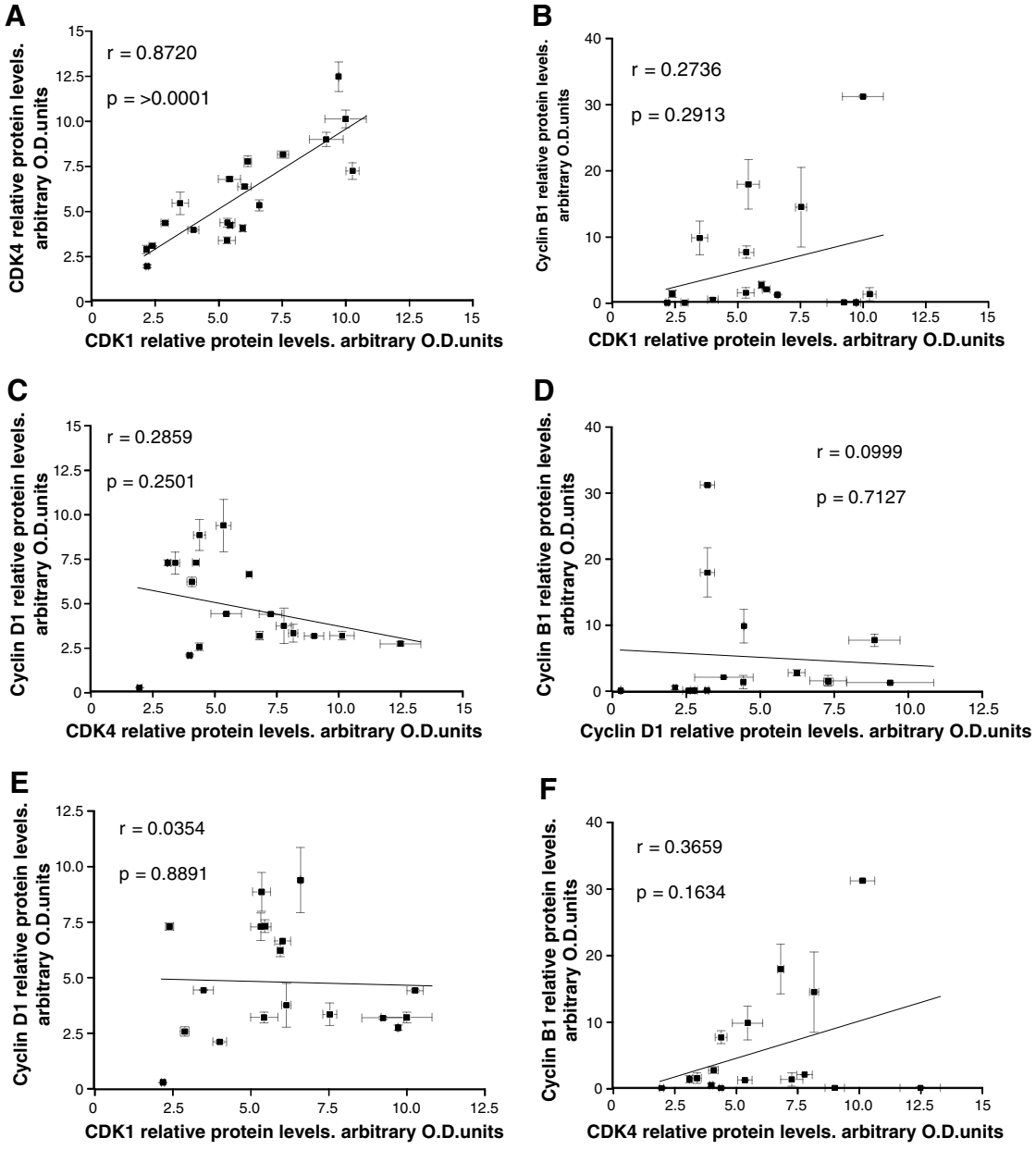

Fig. 2 – Linear regression analysis of the relative protein levels of Cdk1, Cdk4, cyclin B1 and cyclin D1 measured in a range of 20 human *in vitro* cell lines. Proteins were measured by quantitative Western blotting and expressed as relative expression values per 150 μg of total cellular protein as described in Section 2.

transfected, full length wild-type *CDK4* in a pcDNA3.1 plasmid and selected in geneticin. Confirmation of positive transfectants was confirmed by Southern blotting and positive gene expression by Northern blotting (Courtesy of Dr. Roger Barraclough, University of Liverpool, School of Biological Sciences, Cancer and Polio Research Laboratories). Cdk1 and Cdk4 proteomic expression in the transfected RAMA 37 clones was tested by Western blotting as above.

3. Results

3.1. *Confirming the specificity of anti-Cdk1 and anti-Cdk4 antibodies*

The specificity of the antibodies used to measure the proteomic expression of Cdk1 and Cdk4 was checked on Western blots of Cdk1-GST and Cdk4-GST fusion proteins and protein lysates of the 2780 human ovarian cancer *in vitro* cell line.

3.2. *Standardising quantitative Western blotting*

Titration curves confirmed that linear relationships for optical density (OD) could be obtained over the ranges found for Cyclin D1, Cyclin B1, Cdk1 and Cdk4 proteins across the cell lines. In addition, there was a strong linear correlation between cell numbers and total cellular protein when compared by Western blotting.

3.3. *Western blots for Cdk1 and Cdk4*

Typical Western Blots for Cdk1 (cdc2) (panel A) and Cdk4 (panel B) are shown in Fig. 1. The 21 samples comprised the immortalised human ataxia telangiectasia fibroblast cell line, AT5 and human embryonic intestinal cells I407, 18 established human *in vitro* cancer cell lines (see Table 1.) and lysates of normal diploid human keratinocytes (K*) grown in short term primary cultures.

In the majority of cases, expression of both Cdk1 and Cdk4 was higher in the human cancer cell lines than in normal keratinocytes or the immortalised AT5 transformed human fibroblast cell line. Some lanes (viz. 5–7 and 13–17) showed obviously greater expression of Cdk1/Cdk4 than normal keratinocytes, whilst other lanes expressed levels of Cdk1/Cdk4 close to normal cells.

3.4. *Comparison of Cdk1 and Cdk4 proteomic expression in 19 human in vitro cancer cell lines*

Normalised data from the Western blots for Cdk1 and Cdk4 depicted in Fig. 1A and B were independently ranked in the order of ascending intensity of staining (Fig. 1, panels C and D). Linear regression analysis was carried out to compare the relative expression of Cdk1 and Cdk4 in each cell line. A highly significant ($p = 0.0001$) co-relationship between the proteomic expression of Cdk1 and Cdk4 was apparent ($r = 0.87$, $p = 0.0001$) (Fig. 2, panel A). The relative expression of each protein was closely comparable in each cell line across an approximately 5-fold range of Cdk1/Cdk4 proteomic expression.

3.5. *The co-relation between Cdk1 and Cdk4 proteomic expression in human cancer cells is not seen with Cyclin B1, Cyclin D1 or Cdk2*

Similar co-relationships to that of Cdk1/Cdk4 were sought for Cdk2, Cyclin D1 and Cyclin B1 amongst the human cancer cell lines. Fig. 2 demonstrates that in the range of human cancer cell lines which we examined, only Cdk1 and Cdk4 showed related proteomic co-expression. Cdk2, Cyclin D1 and Cyclin B1 were not related to one another or to Cdk1 and/or Cdk4.

3.6. *The co-relation between Cdk1 and Cdk4 proteomic expression in human cancer cells is not seen in normal fibroblasts or keratinocytes*

When the relative proteomic expression of each of Cdk1 and Cdk4 was compared in four independent primary cultures of explanted normal human fibroblasts and eight independent primary cultures of human keratinocytes, the two cyclin-dependent kinases showed no co-related expression (Fig. 3).

3.7. *Protein half-lives of Cdk1 and Cdk4 in human cancer cell lines*

In seeking a potential mechanism for Cdk1/Cdk4 proteomic co-expression, the protein half-lives of both cyclin-dependent kinases were measured in four human cancer cell lines (HT29.5, HX142/61, MGHU1 and RTII2) (Fig. 4) chosen because they covered the range of Cdk1/Cdk4 expression across the 18 human *in vitro* cancer cell lines. Whilst there was a significant correlation between Cdk1 and Cdk4 half-lives in each of the cell lines ($r = 0.97$, $p = 0.03$) (Fig. 5, panel C), the overall levels of expression of Cdk1 and Cdk4 were independent of the rates of protein degradation (Fig. 5, panels A and B).

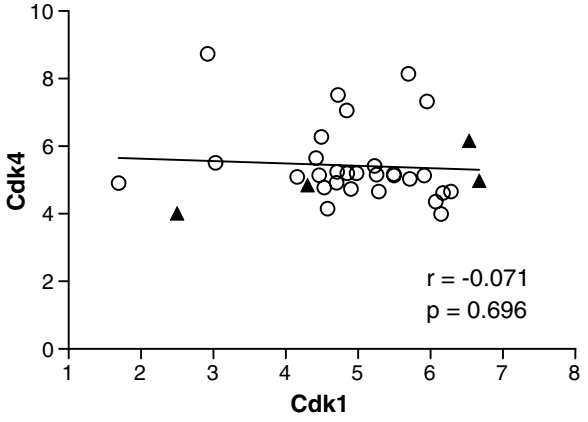

Fig. 3 – Cdk1 and Cdk4 relative protein expression in four short term cultures of normal human diploid fibroblasts (▲) and eight short term cultures of normal human diploid keratinocytes (○). Pooled data from 33 independent measurements.

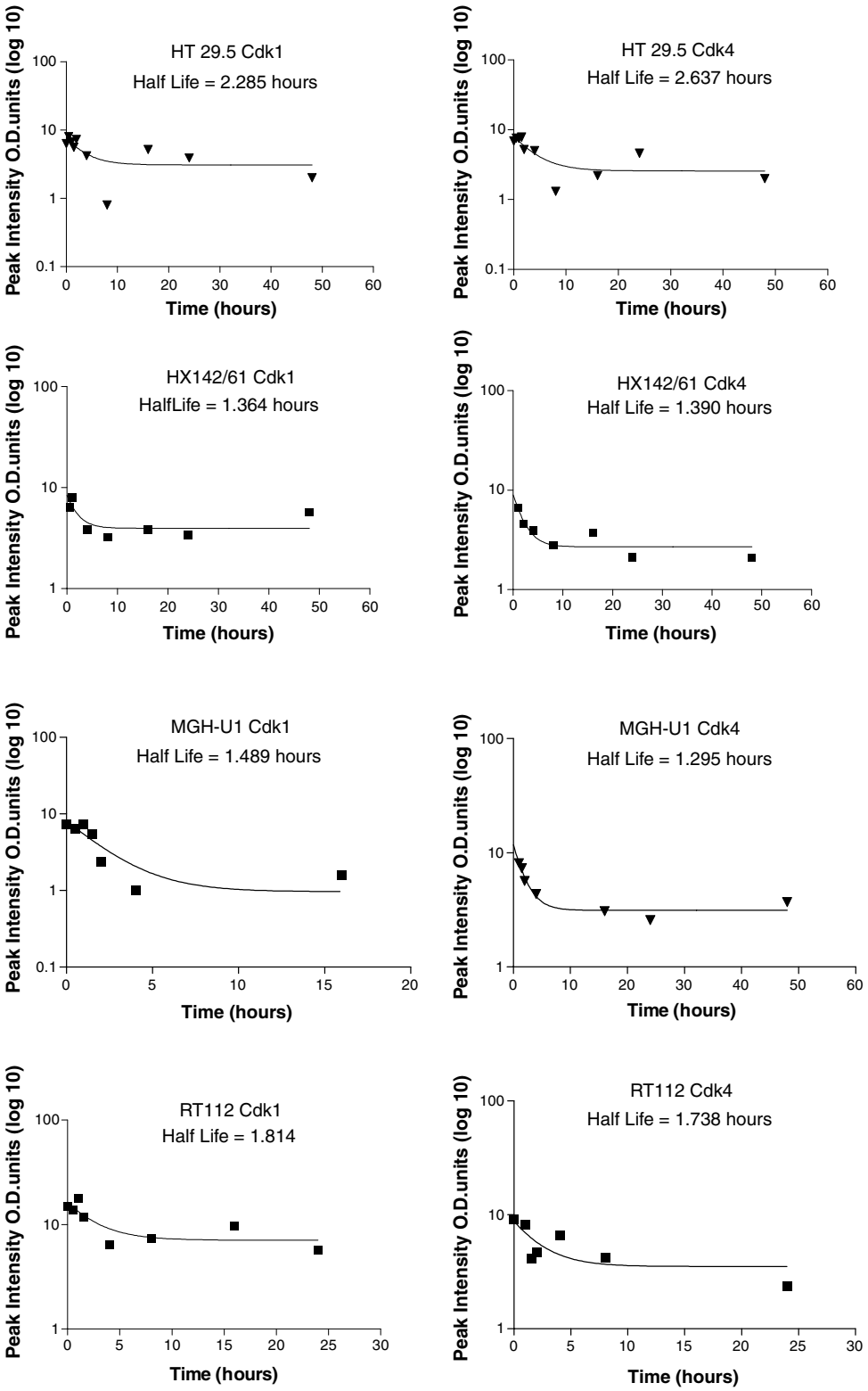

Fig. 4 – Protein half-lives of Cdk1 and Cdk4 in the four human cancer *in vitro* cell lines. HT29.5, HX142/61, MGHU1 and RT112. Exponential growing phase cells were incubated in the presence of depleted medium (minus methionine) for 1 h at 37 °C, then labelled with 250 μCi/ml ^{35}S-Methionine for 45 min at 37 °C. Each flask was then chased with medium containing 100 times excess cold methionine. Each lysate was immunoprecipitated followed by SDS–PAGE on a 12% Laemelli discontinuous gel. The film was processed as for ECL detection and the banding patterns were examined on gel analysis software (Phoretix). The values produced were analysed by a statistical package (Prism 2.01).

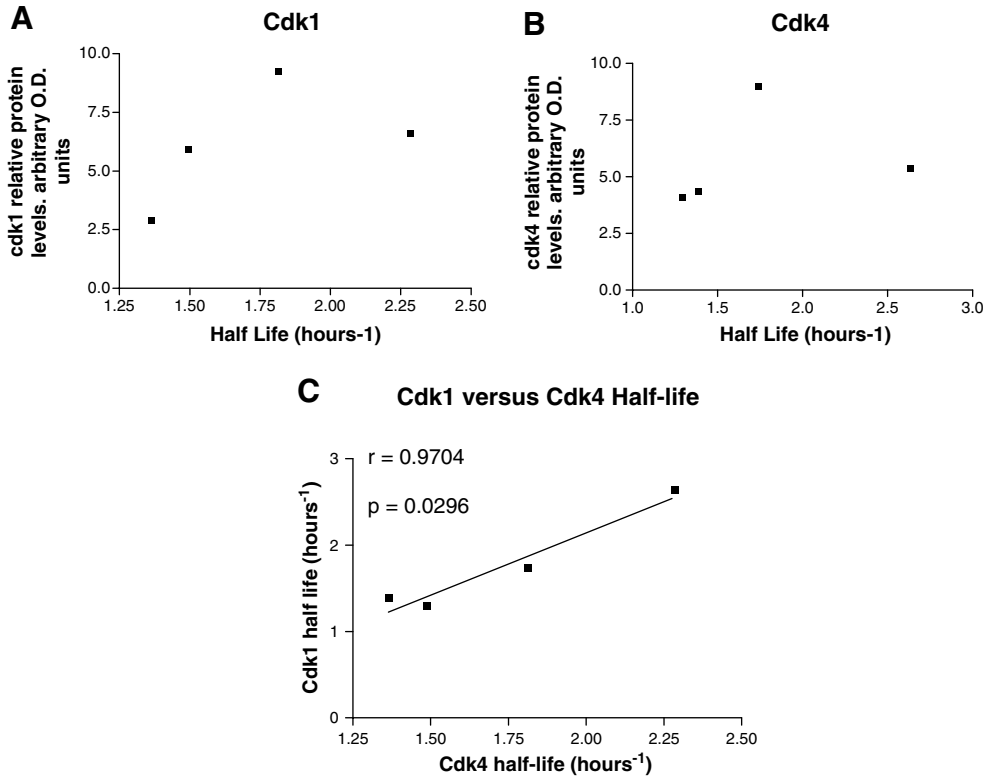

Fig. 5 – Comparison of Cdk1 and Cdk4 protein half-lives. Linear regression analysis of the relative protein expression levels of Cdk1 and Cdk4 at time zero plotted against their respective half-lives in four human *in vitro* cell lines (A and B). Linear regression analysis of the relationship between the protein half-lives of Cdk1 and Cdk4 for the four cell lines (C).

3.8. *Transfection of RAMA 37 mammalian cells with CDK1 and CDK4 cDNA*

In order to determine whether there might be a causal relationship between the co-expression of *CDK1* and *CDK4*, full length normal cDNA transcripts of the *CDK1* and *CDK4* genes were independently transfected into the established RAMA 37 rat mammary epithelial cell line. In the case of both *CDK4* and *CDK1*, the transfectants grew well and the clones were easily selectable following 2 weeks exposure to geneticin. *CDK1* transfectants yielded 10 positive clones in which Southern blotting identified the transfected *CDK1* pcDNA3.1. Similarly, *CDK4* yielded eight positive clones following geneticin exposure. However, whilst all eight *CDK4* transfectants were positive for gene expression on Northern blotting, none of the *CDK1* transfectants showed any increased RNA transcript above base line. Western blotting for Cdk1 and Cdk4 proteins was carried out on the positive *CDK4* transfected clones. An approximately 5-fold range of Cdk4 proteomic expression was seen across the transfected clones (Fig. 6), which was accompanied by a highly significant concomitant increase of proteomic expression of Cdk1 ($r = 0.88$, $p = 0.004$).

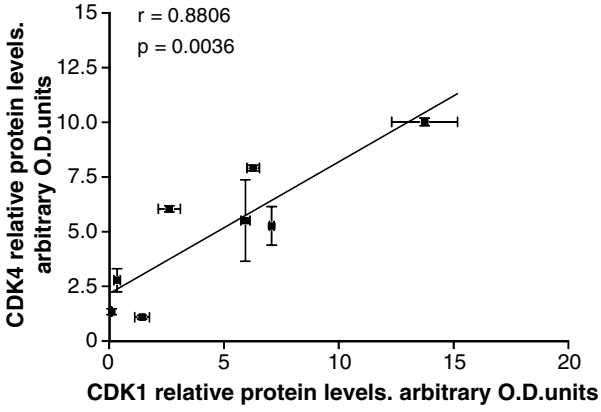

Fig. 6 – Western blot analysis of lysates from RAMA 37 mammalian cells transfected with Cdk4. Linear regression analysis of the relative protein levels of Cdk1 against Cdk4 following Western blot analysis. Seventy-five micrograms of total cellular protein obtained from Cdk4 transfected RAMA 37 cells was loaded onto a 12% Laemelli discontinuous gel and analysed by 12% SDS–PAGE, detected by ECL and examined using gel analysis software (Phoretix). The means of quadruplicate values were calculated and used to measure linear regression resulting in the correlation coefficient (*r*) and the degree of significance (*p*) for the Cdk1 and Cdk4 relationship (Prism 2.1).

3.9. *Cdk1/Cdk4 co-expression in clinical metastatic malignant melanoma*

Tang and colleagues[19] have previously reported a quantitative Western blot study of the proteomic expression of cyclins and their dependent kinases in 10 samples of metastatic malig-

nant melanoma. Re-analysis of the Tang data in Table 1 of their paper by our method is shown in Fig. 7. A significant co-relationship between cyclin A and CDC25A as described by Tang and colleagues in their analysis of the data was identified by our own method of analysis ($r = 0.682$, $p = 0.03$) (Fig. 7A). In addition, a similar pattern of Cdk1/Cdk4 protein co-expression ($r = 0.82$, $p = 0.004$) to that seen in our *in vitro* cancer cell lines can be seen across the range of the 10 different clinical biopsies (Fig. 7B).

4. Discussion

In this study, we have identified a co-relationship between Cdk1 and Cdk4 protein expression in human cancer cell lines that is not present in normal human diploid cells grown in short term culture.

In order to compare the abundance of different proteins, we normalised and ranked their relative cellular expression, enabling direct comparison to be made between any two or more protein profiles irrespective of their immunochemical staining characteristics or absolute cellular levels. For some cancer cell lines, Cdk1 and Cdk4 expressions are low and close to that of normal cells. In other cases, there is a 5-fold greater protein abundance of Cdk1 and Cdk4. Our analyses provide a ratio of Cdk1 to Cdk4 in individual cancer cell lines across the whole range of proteomic expression of these two cyclin-dependent kinases. The ratios of Cdk1 to Cdk4 in cancer cells (mean 1.014, range 0.6–1.4) (Fig. 8) show a tight distribution compared to normal human diploid fibroblasts and keratinocytes growing in primary cultures, where a wide spread of Cdk1/Cdk4 ratios with a less obvious peak is seen.

We applied our method of analysis to the quantitative Western blotting data of Tang and colleagues[19]. In addition to confirming their observation that Cdc25A and Cyclin E are co-related, we also show that Cdk1 and Cdk4 proteins are co-expressed in a clinical cancer as well as in human cancer cell lines.

Proteomic expression of cyclin-dependent kinases is generally understood to be relatively constant throughout the cell-cycle and controlled by transcription.[25,26] Cdks can only influence cell-cycle progression when activated by forming holoenzymes with their relevant cyclin partners. In human

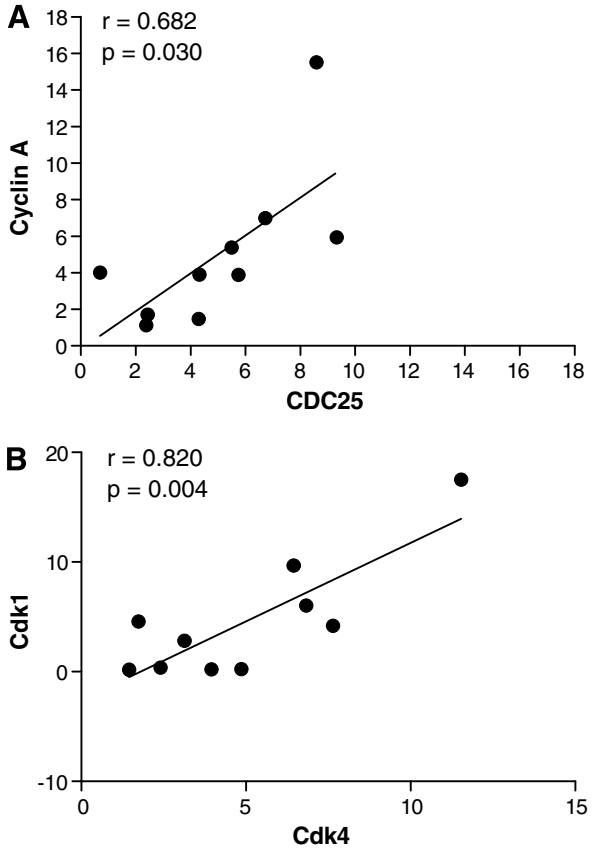

Fig. 7 – Linear regression analysis of proteomic expression in human malignant melanoma. Significant co-expression can be seen for cyclin A/CDC25 (A) and Cdk1/Cdk4 (B). Data were re-analysed from Tang et al.[19] (see Section 2).

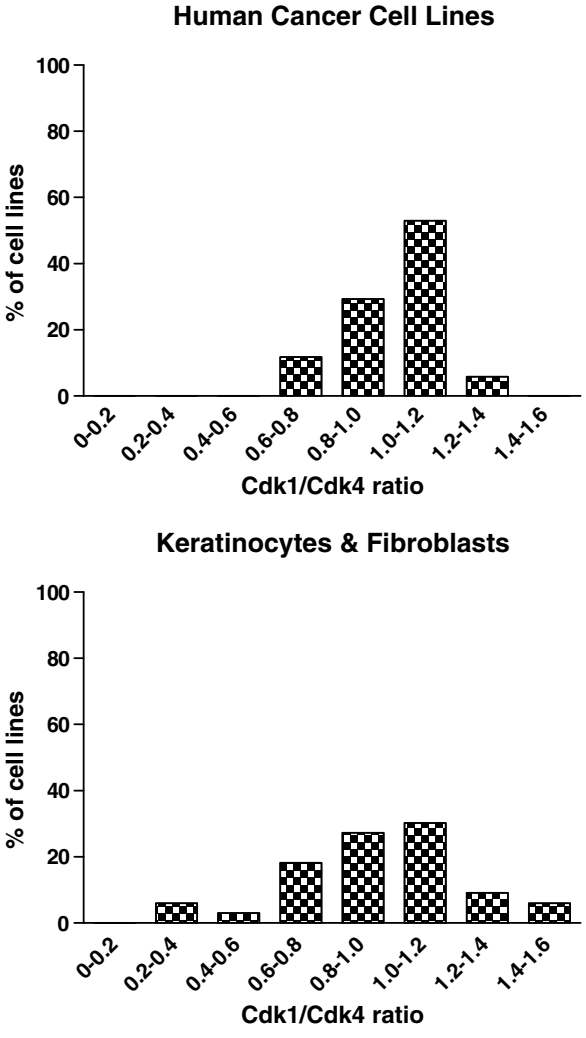

Fig. 8 – Comparison of Cdk1/Cdk4 ratios in human cancer cells and normal diploid keratinocytes and fibroblasts. The ratios of Cdk1/Cdk4 in 18 human cancer cell lines, 33 data points from eight independent short term primary cultures of normal diploid keratinocytes and four independent short term primary cultures of normal diploid fibroblasts (see Fig. 3). Ordinate Cdk1/Cdk4 ratios. Abscissa percentage of cells expressing this ratio.

cancer cells we have found variable expression and a co-relationship of Cdk1 and Cdk4 proteins alone. We investigated the Cdk1/Cdk4 protein half-lives in four cell lines chosen to span the range of Cdk protein expression. The protein half-lives of Cdk1 and Cdk4 in each cell line were closely significantly related ($r = 0.970$, $p = 0.030$).

In attempting to determine whether the co-expression of Cdk1 and Cdk4 reported here reflected a causal relationship, rat mammary epithelial cells (RAMA 37) were transfected with full length normal Cdk4 cDNA. Increased expression of Cdk4 protein was found to be accompanied by concomitant elevation of endogenous Cdk1. Taken with the protein half-life data above, these transfection experiments indicate that Cdk4 can induce Cdk1 expression but the similar levels of the Cdk1 and Cdk4 proteins are, at least in part, a result of co-ordinated protein breakdown.

Sequencing of cDNA in the human *in vitro* cancer cell lines described here has shown both CDK1 and CDK4 to be wild-type in all cell lines (unreported data). It has previously been postulated that certain critical normal genes may need to be retained throughout the life of progressively evolving cancer cells to enable them to continue to divide successfully.[27] Because their continued expression is mandatory for cell survival, loss or functional disruption of the products of such genes would result in cell death. Unlike the majority of anti-cancer drug targets, such normal genes would not be subject to heterogeneity which is a frequent source of drug resistance. Cdk1 and Cdk4 may be acting co-operatively as critical normal gene products in the above way.

Over a 5-fold range, Cdk4 and Cdk1 proteins can be seen to be co-ordinately expressed at discrete levels in individual cancer cells both in laboratory cell lines and in the clinic. In addition, overexpression of Cdk4 as the result of transfection results in increased expression of Cdk1. We suggest that the reason that the human cancer cells, reported here, exhibit a Cdk1/Cdk4 ratio close to unity could be that Cdk4 stimulates an increase in Cdk1 which itself acts via, an as yet unknown, negative feedback loop to depress Cdk4. If this were the case, overexpressed Cdk1 protein on its own would act to diminish endogenous Cdk4 levels in the pcDNA3 *CDK1* transfectants and inhibit cell proliferation. This could explain our failure to obtain any clones with positive expression of Cdk1 despite repeated *CDK1* DNA transfection experiments.

Cdk4 with its cyclin D partners initiates the molecular processes, which begin cell division, by phosphorylating the retinoblastoma protein and associated pRb family members[6,7] and liberating E2F factors.[28] Activation of the Rb/E2F pathway, however, may not only increase cell proliferation but may also result in apoptosis.[29] To be successful, cancer cells must be able to maximise proliferation whilst avoiding apoptosis. Prolonged, selective small-molecule inhibition of Cdk1 has recently been shown to induce apoptosis which was more marked in human cancer cells than non-tumourigenic cells,[30] suggesting that Cdk1 expression may normally have a therapeutically exploitable, anti-apoptotic action, in human cancer. We suggest that in addition to initiating cell proliferation through the Rb/E2F pathway, Cdk4 may also minimise the potential resulting apoptosis by direct stimulation of Cdk1. Disruption of the Cdk1/Cdk4 relationship might thus act therapeutically by directing cancer cells towards apoptosis rather than proliferation.

Over the past decade, the roles of cyclins and their dependent kinases in normal and cancer cells have undergone re-evaluation.[11,15,16] Deshpande et al. have emphasised that there is a need to rethink the existing models of cyclin and cyclin-dependent kinase function.[12] The findings presented in this paper show a novel phenomenon common to a broad range of human *in vitro* cell lines of many different histogenetic origins which is also identifiable in metastatic clinical cancer. We suggest that tighter control of the levels of Cdk1 and Cdk4 in cancer cells may represent a control mechanism that enables the cancer cell to relentlessly proliferate whilst avoiding apoptosis. In such a situation, disruption of Cdk1/Cdk4 co-expression might potentially have a selective anti-cancer effect.

Conflict of interest statement

H.M. Warenius is co-founder and Director of R&D, TheRyte Ltd.

Acknowledgements

We are very grateful to Carole Thomas for excellent technical assistance, particularly in maintaining stocks of high quality, contaminant-free primary culture and established cell lines. We also thank Lito Kyritsi and Sophy Moumtzi for contributing to Cdk1/Cdk4 Western blotting data in normal cells, Tracy Gorman for preparing and transfecting wild-type Cdk4 cDNA3 plasmids under Dr. Roger Barraclough's supervision and Amanda Howarth for collating and analysing data and preparing scientific tables and figures.

This work was supported by a grant from the Cancer and Polio Research Fund, Hoylake, Wirral, UK.

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