cDNA analysis of gene expression associated with DNA-dependent protein kinase activity

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Abstract. DNA-dependent protein kinase (DNA-PK) is thought to play a pivotal role in DNA double-strand break repair. We recently demonstrated the association of DNA-PK activity in peripheral blood lymphocytes (PBL) with the incidence of chromosomal aberrations and the risk of cancer. In this study, we applied cDNA array technology to find the expression of genes which are associated with DNA-PK activity in PBLs with various levels of DNA-PK activity. Most genes correlated with DNA-PK activity involved cell cycle regulation. Moreover, the transcription factor E2F1, which plays an important role in cell cycle progression, exhibited strong correlation with the DNA-PK activity and Rbp130, which is considered a negative regulator of E2F, showed inverse correlation with DNA-PK activity. *In silico* promoter analyses showed the presence of at least one E2F binding site in the promoter regions of Ku70, Ku86, DNA-PKcs and genes associated with DNA-PK activity. In order to examine the relationship among the E2F1 expression, the expression of genes related with DNA-PK activity, and DNA-PK activity, we activated PMLs by PHA to progress the cell cycle. After PHA activation of PML, the expression of E2F1 and DNA-PK activity increased. The expression of most genes in PHA-stimulated PBLs had a similar relationship with DNA-PK activity to that without PHA stimulation. These results indicate that the E2F transcription factor may regulate the concerted expression of genes related with DNA-PK activity.

Introduction

Repair of various types of DNA damage is critical for cell survival. DNA double-strand break (DSB) is believed to be one of the most serious damage induced by DNA damaging agents such as ionizing irradiation (1) and, unless repaired properly, it can lead to cell death during mitosis or accumulation of damage, which, in turn, induces genetic instability and neoplastic transformation (2).

In DNA DSB repair, at least two major repair mechanisms, homologous recombination (HR) and non-homologous end-joining (NHEJ) have been reported (3). In NHEJ pathway which is the major mechanism in mammalian cells, DSBs are directly, or after processing of the DNA ends, rejoined at an appropriate chromosomal end and DNA-dependent protein kinase (DNA-PK) plays an important role in DNA DSBs repair by NHEJ throughout the cell cycle (4). DNA-PK is a serine/threonine kinase, which is composed of DNA-PK catalytic subunit (DNA-PKcs) and the heterodimers Ku70 and Ku86. DNA-PK binds DSBs in DNA, phosphorylates and activates DNA-binding protein, including XRCC4 and DNA ligase IV, p53, and several transcription factors. Subsequently, Ligase IV repairs DNA DSB (5). This DNA repair pathway has been implicated in maintaining genomic integrity via suppression of chromosomal rearrangements (3,6,7).

In a previous study, we demonstrated that DNA-PK activities of peripheral blood lymphocytes (PBL) in cancer patients were significantly lower than those in normal volunteers (8). The frequency of chromosome aberration increased as the DNA-PK activity decreased. Therefore, DNA-PK might function as a cancer susceptibility gene. Moreover, the results of RT-PCR experiments indicated that DNA-PK activity correlated with expression of Ku70, Ku86, and DNA-PKcs, which are interrelated with one another. Similar tendency was observed also at protein level in Western blot assay. These results in the aggregate suggested a possibility that the activity of DNA-PK may be regulated at the mRNA level and the mRNA expression of DNA-PKcs, Ku86 and Ku70 may be, at least partially, coordinated.

cDNA array technique enables robust analyses of the expression of a number of genes in a single experiment. To approach the mechanism regulating the expression of DNA-PK subunits, we performed a genome-wide search for genes whose expression is correlated with DNA-PK activity, using cDNA array. We also investigated the mechanisms of the

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concerted expression of these genes that were related with DNA-PK activity.

Patients and methods

Selection eligibility. All subjects were Japanese. They consisted of five cancer-free normal healthy volunteers and five cancer patients; two sporadic breast cancer patients, one hypopharyngeal cancer, and two uterine cervix cancer. Exclusion criteria included a history of other cancer, chemotherapy, radiation therapy, or current use of immunosuppressive medications. The study was approved by the appropriate Committees for Human Rights in Research in our hospital and written informed consent was obtained from each subject.

Blood collection and PBL separation. Peripheral blood (20 ml) was collected from all subjects with a sterile heparinized tube. PBLs were separated with lymphoprep (Nycomed Pharma AS, Oslo, Norway), centrifuged at 1500 rpm (300 x g) for 30 min at 4˚C, and washed twice with PBS (phosphate-buffer saline).

PBLs were incubated in 10 ml of RPMI-1640 medium (Sigma Aldrich, Taurtkirchen, Germany), supplemented with 20% fetal calf serum. When PHA was used to stimulate proliferation of PBLs, PHA (Murex, Dartford, UK) was added at 1% of final concentration for 48 h. After 48 h, PBLs were washed twice with PBS and used for DNA-PK assay (or mRNA extraction).

PBL lysis, protein extraction, concentration assay. PBL was thawed with high salt buffer (20 mM HEPES-KOH (pH 7.9), 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 0.02% Tween-20, 10% glycerol, 1 mM DTT, 1 mM PMSF, 1 μg/ml leupeptin, pepstatin, antipain, respectively), and the suspension was lysed by three rounds of freeze-thaw cycle, i.e. repeated freezing in liquid nitrogen bath followed by thawing in water bath at 30˚C, and clarified by centrifugation at 15,000 rpm (18,000 x g) for 7 min at 4˚C. Protein concentration was assayed using a BCA protein assay kit (Pierce, Rockford, IL, USA) with bovine serum albumin as the standard.

The assay procedure for DNA-PK activity has been described in our earlier report (8). The PBL cell lysates were diluted to three protein concentrations (0.5, 0.25, 0.125 mg/ml, respectively) with high salt buffer. The diluted lysate (5 μl) was mixed with 15 μl of 1.33X kinase assay buffer (contents of 1X kinase assay buffer: 20 mM HEPES- NaOH (pH 7.2), 5 mM $MgCl_2$, 150 mM KCl, 50 μM [γ-^{32}P]-ATP, 1 mM DTT, 0.5 mM NaF and 0.5 mM ß-sodium glycerophosphate), 0.25 μg/μl synthetic peptide hp53-S15 (sequence: EPPLSQEAFADLWKK; synthesized in Sawady Biotechnology, Tokyo, Japan), and with or without 20 ng/μl sonicated salmon sperm DNA. This reaction mixture was incubated at 37˚C for 10 min. The reaction was stopped by the addition of 20 μl of 30% acetic acid and absorbed onto a phosphocellulose filter disc (2.3 cm in diameter, Whatman, Maidstone, UK). The filter discs were washed in 15% acetic acid and in 99% ethanol and the remaining radioactivity was counted in a liquid scintillation counter. The net phosphorylation of hp53-S15 was calculated as phosphate incorporation in reaction with DNA minus that in reaction without DNA, divided by the specific radioactivity of ATP. In this study, DNA-PK activity was expressed as the amount of ATP (unit is 'pmol') used for DNA dependent phosphorylation of hp53-S15.

Semiquantitative RT-PCR. We selected 8 individuals who had various activities of DNA-PK for semiquantitive RT-PCR assay. Total RNA isolated from PBLs of each individual using Triazol reagent (Life Technologies, Rockville, MD) was used for mRNA extraction using a MagExtractor mRNA isolation kit (Toyobo, Tokyo, Japan) according to the manufacturer's protocol. The mRNA samples were used to synthesize the first strand cDNA (Life Technologies). PCR was performed using primers specific for Ku70 and Ku86, and GAPDH genes in duplex PCR reactions. GAPDH served as an internal control of the reaction. Results were analyzed using a multi-image analyzer (Bio-Rad).

Analysis of cDNA expression using cDNA array filters. The same mRNA samples used for RT-PCR assay were used for cDNA array analysis. Biotin-labeled cDNA targets were made from 3 μg of total RNA using Gene Navigator cDNA amplification system (Toyobo), including random 9 mer, biotin-16-dUTP, and ReverTraAceTM reverse transcriptase. Free biotin-16-dUTP in the reaction was removed by ethanol precipitation. Gene Navigator cDNA array filter (human cancer, Toyobo) consisted of 536 cancer-related genes and 26 housekeeping genes in duplicate. A complete list is available on the internet (http://www.toyobo.co.jp). Hybridization was performed overnight at 68˚C in PerfectHyb™ (Toyobo). Filters were washed 3 times in 2X SSC/0.1% SDS at 68˚C for 10 min each, followed by 3 washes in 0.1X SSC/0.1% SDS at 68˚C for 10 min each. Specific signals on the filters were detected by a chemiluminescence detection kit (Imaging high™, Toyobo). CDP-Star was used as the chemiluminescent substrate. Quantitive assessment of the signals on the filters was performed by scanning on a Fluor-S Multi-imager System (Bio-Rad, Richmond, CA) followed by image analysis using ImaGene software (BioDiscovery, Los Angeles, CA). The data were analyzed by normalization to GAPDH expression.

In silico promoter analyses. In general, the genomic sequence of 2-kb upstream of the initiation codon was retrieved via NCBI website (http://www.ncbi.nlm.nih.gov/) and analyzed using a program 'TFSEARCH: Searching Transcription Factor Binding Sites', which is written by Yutaka Akiyama (Computational Biology Research Center, Tsukuba, Ibaraki, Japan) and is available at the website http://www.rwcp.or.jp/papia/ (9).

Statistical methods. Spearman's rank correlation test was used to compare DNA-PK activity and expression of various genes in RT-PCR and cDNA array.

Results

First, in order to verify cDNA array results, expression of Ku70 and Ku86 was examined by semiquantitative RT-PCR using the same mRNA samples that had been used for the array analysis. Semiquantitative RT-PCR analysis of these

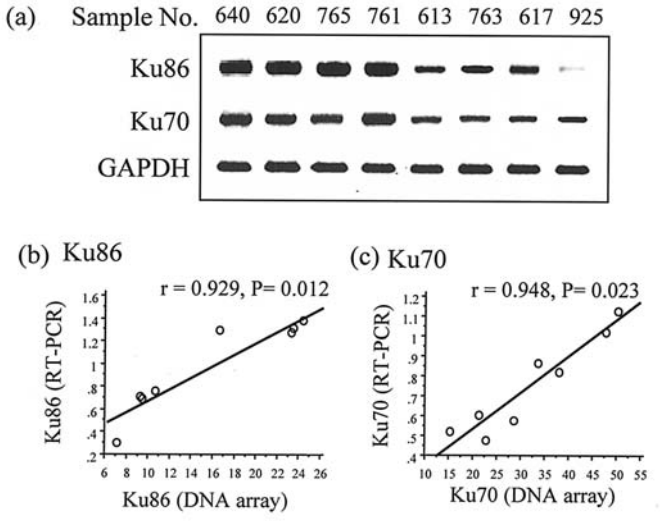

Figure 1. (a) The expression of Ku70, Ku86, and GAPDH in 8 individuals who had various activities of DNA-PK. The expression of mRNA was analysed by semiquantitative RT-PCR. Expression of Ku 70 (b) and Ku86 (c) identified by cDNA array analysis was examined by semiquantitative RT-PCR using the same mRNA samples that had been used for the array analysis.

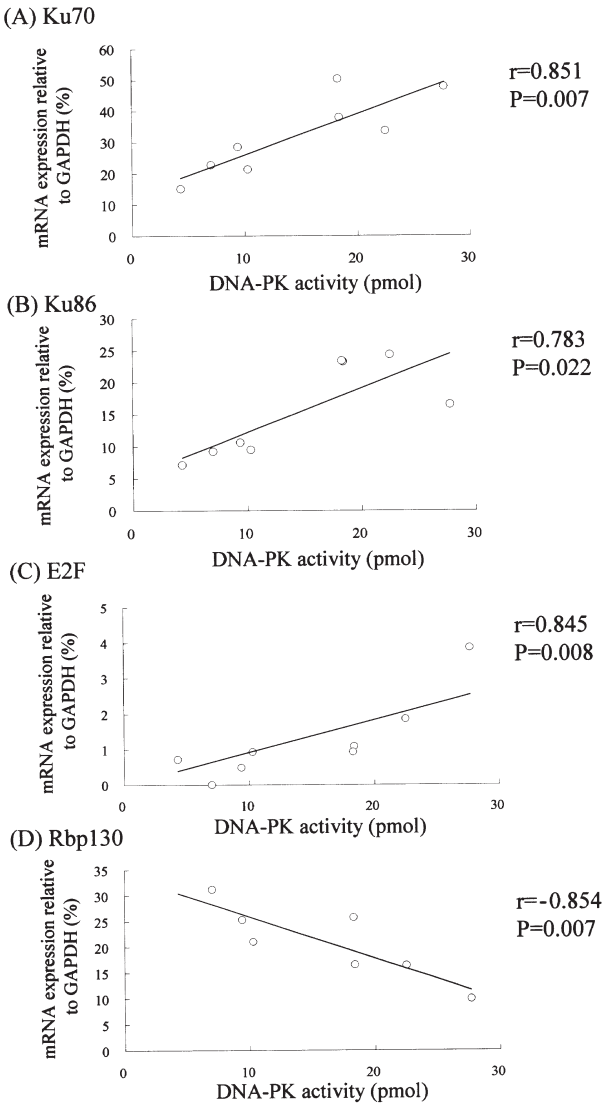

Figure 2. The relationship between DNA-PK activity and expression of genes which were positively or negatively correlated with DNA-PK activity. The expression level of these proteins is indicated by values relative to GAPDH.

differentially expressed genes gave results consistent with those by cDNA array analysis (Fig. 1).

To identify genes whose expression correlated with DNA-PK activity, we analyzed results of cDNA array which were performed by using PBLs with varying levels of DNA-PK activity. We compared expression of various genes with DNA-PK activity and calculated correlation coefficients between them (Table I).

Among 536 genes that were analyzed, 33 genes exhibited strong correlation with DNA-PK activity, i.e., with the correlation coefficient greater than 0.7 (Table I and Fig. 1). Among 33 genes, 32 (e.g. PCNA, Rap 1B, XRCC3) were positively correlated with DNA-PK activity, and only one (Rbp130) was correlated negatively (Fig. 2). Not surprisingly, Ku70 and Ku86, which are subunits of DNA-PK, were included in the former group. We performed clustering analysis of these 33 genes (Fig. 3). As expected, Rbp130 was separated from the other 32 genes. Furthermore, Ku70 and Ku86 fell into the neighboring cluster, suggesting that the expression of these two genes were governed, at least partially, by a common regulatory mechanism. DNA-PKcs was not on the array used here. Interestingly, a substantial portion of the genes found, i.e., PCNA, E2F1, cyclin D1, cyclin E, p55 CDC, Ki-67, Ran and cyclin D3, are implicated in cell cycle progression, while Rbp130 is a negative regulator of cell cycle progression.

E2F family proteins, via dimerization with DP proteins, facilitate the transcription of many proteins with supposed functions in cell cycle progression and DNA synthesis/replication (10,11). On the other hand, Rb family proteins (p110, p107 and p130) are thought to exert their growth suppressive activity by binding to E2F family proteins and then inhibiting the transcription of E2F target genes. Although it was initially thought that the main function of E2F might be cell cycle progression and DNA replication, accumulating evidence indicates that E2F is also important for DNA repair, check-point, cell differentiation and apoptosis. Additionally, recent studies employing cDNA array, sometimes in combination with chromatin immunoprecipitation, revealed a number of new E2F target genes, which include not only cell cycle- or DNA replication-related genes but also those related to DNA repair, check-point, cell differentiation and apoptosis (12-19). The above results led us to consider the possibility that the expression of DNA-PK subunits and correlated genes might have been modulated by the E2F-pRB system. Among 33 genes found above, cyclin D1, D3, E, PCNA, p55 CDC, Ki-67 and E2F1 itself have been listed as E2F target in earlier studies. Additionally, one study showed that DNA-PKcs is also one of the E2F targets (15).

To explore the possibility that genes found above are also regulated by the E2F-Rb system, we performed *in silico* promoter analyses, looking for potential E2F binding sites (Fig. 4). Out of 22 genes examined, 19 (except for PCNA, Rap1A and ENT1) bore at least one E2F binding site within the 2-kb region upstream of the respective translation initiation sites (denoted +1 ATG in Fig. 4). Although E2F binding site

Table I. Gene expressions related with DNA-PK activity.

Gene name	Accession no.	Relative coefficiency DNA-PK activity	P-value DNA-PK activity	Relative coefficiency E2F1 expression	P-value E2F1 expression
PCNA (proliferating cell nuclear antigen)	M15796	0.938	0.0006	0.88	0.0039
Rap 1B	X08004	0.869	0.0051	0.586	0.1272
Rad9	U53174	0.864	0.0057	0.873	0.0047
XRCC3 (X-ray repair cross-complementing protein 3)	AF035586	0.863	0.0057	0.812	0.0145
Ku70	J04607	0.851	0.0074	0.532	0.1224
SUMO	U72722	0.85	0.0076	0.749	0.0323
E2F1	M96577	0.845	0.0083	-	-
cyclin D1 (CYL1)	X59798	0.83	0.0271	0.367	0.3706
MFGE8 (milk fat globule-EGF factor 8 protein)	U58516	0.819	0.0218	0.604	0.1124
PDGF b	X02811	0.808	0.152	0.95	0.0003
Cyclin E	M73812	0.802	0.0165	0.587	0.1257
hRAD50	Z75311	0.795	0.0185	0.587	0.1257
Ku80	M30938	0.783	0.0217	0.367	0.3706
Rap 1A (KREV-1)	M22995	0.782	0.0218	0.604	0.1124
Pim-1	M54915	0.766	0.0267	0.759	0.0291
Thrombospondin 3	L38969	0.765	0.027	0.807	0.0154
ENT1 (es transporter)	AF079117	0.762	0.0281	0.809	0.0149
p55 CDC	U05340	0.761	0.0283	0.917	0.0014
Connexin 32	X04325	0.759	0.0289	0.948	0.0003
PLC-γ1	M34667	0.757	0.0297	0.749	0.0326
Connexin 43	M65551	0.75	0.0321	0.921	0.0011
Ki-67	X65551	0.745	0.034	0.902	0.0022
GAK (cyclin-G associated kinase)	D88435	0.743	0.0345	0.614	0.1051
ARF4	M36341	0.742	0.0349	0.574	0.1366
CAS (cellular apoptosis susceptibility)	U33286	0.741	0.0354	0.753	0.0312
Ran	AF052578	0.729	0.04	0.412	0.3106
GSTT1 (glutathione S-transferase T1)	X79389	0.72	0.0442	0.775	0.0238
Cyclin D3 (CYL3)	M92287	0.718	0.0451	0.771	0.0252
MMP7 (PUMP-1)	Z11887	0.717	0.0453	0.884	0.0036
Integrin α6 (CD49F)	X53586	0.714	0.0465	0.869	0.005
JAK1	M64174	0.704	0.0513	0.476	0.2326
GM2 activator protein	L01439	0.701	0.0527	0.776	0.0236
Rbp130	X76061	-0.854	0.0145	-0.893	0.00675

was not found in this region of PCNA, five sites were found downstream of the translation initiation site, i.e., one in exon 1 and four in intron 1. ENT1 also bore an E2F binding site in intron 1. In Rap1A, an E2F binding site was found near the transcription start site in untranslated exon, which is 29 kb apart from the translation initiation site. Thus, potential E2F binding sites were found in all the genes examined.

In order to examine the relationship among the E2F1 expression, the expression of cell cycle related genes, and DNA-PK activity, we stimulated PMLs by PHA to resume the cell cycle progression. After PHA activation of PML of two individuals, the expression of E2F1 increased 85.5 and 73.7 times after PHA activation. DNA-PK activity also increased 3.29 and 1.61 times, respectively. We also measured DNA-PK activities of PML of 8 individuals and found that it increased 2.56±0.86 times. Table II shows the change of the expression of 33 genes after PHA activation in two individuals. Expression of most genes including Ku70, Ku86, XRCC3, and cyclin D3, increased, but that of Rbp130 and, unexpectedly, cyclin E decreased. Fig. 5 demonstrates how the expression of mRNA of genes that were correlated with DNA-PK activity in non-activated PMLs changed after

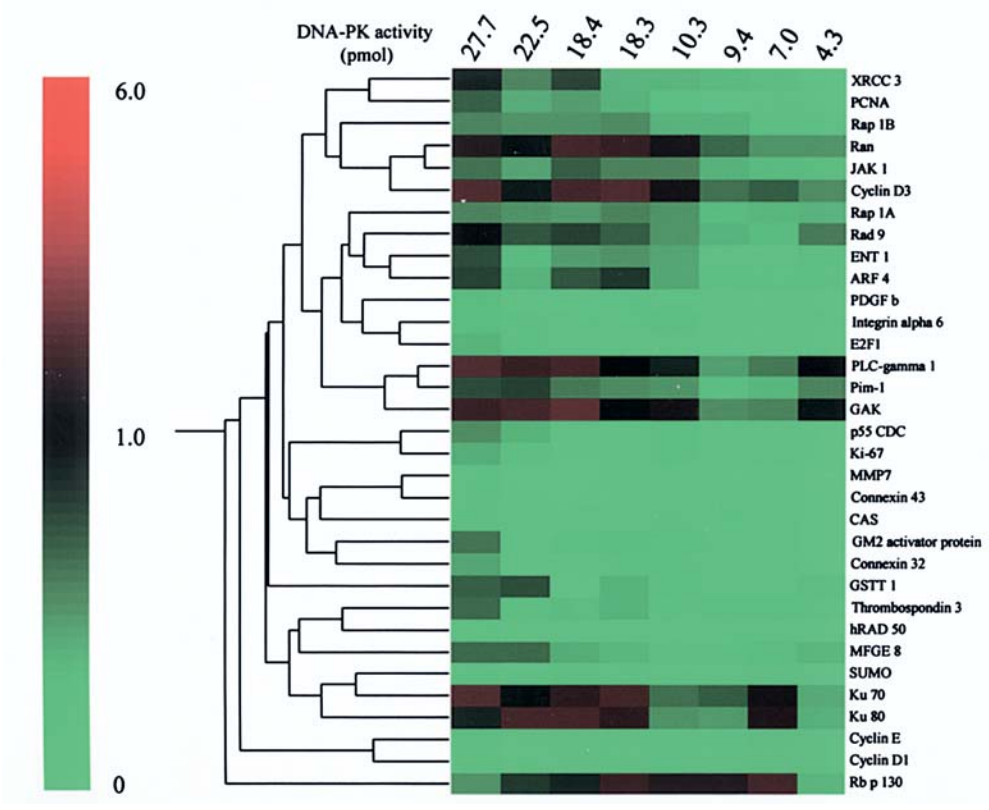

Figure 3. Hierarchical clustering of 33 genes in 8 individuals who had various activities of DNA-PK. The color in each well represents relative expression of each gene (vertical axis) according to DNA-PK activity (horizontal axis). The color scale indicates the ratio of the expression of each gene to that of GAPDH.

activation with PHA. The expression of all genes in PHA-stimulated PBLs had a similar relationship with DNA-PK activity as those without PHA stimulation.

Discussion

In our previous study, we found that DNA-PK activity in PBL varied by a factor of 10 among the individual subjects (8). DNA-PK activities of PBL in cancer patients were significantly lower than in normal volunteers. Even among normal volunteers, there is 2.6 times difference in the activity of DNA-PK. Besides, the frequency of chromosome aberration such as dicentric chromosomes and excess fragment increased as the DNA-PK activity decreased. Therefore, DNA-PK might function as a cancer susceptibility gene. Age and smoking had no association with DNA-PK activity. There was a relationship between DNA-PK activity and expression of Ku70, Ku86, and DNA-PKcs in RT-PCR assay, indicating that the difference of DNA-PK activity may be due to the different expression of these proteins that are constituents of DNA-PK.

Then, cDNA array analysis was performed to find proteins other than Ku70, Ku80, and DNA-PKcs which influence DNA-PK activity. We also performed semiquantitative RT-PCR analysis of Ku70 and Ku86 to confirm the credibility of the cDNA array. Results of RT-PCR analysis were consistent with those of cDNA array analysis (Fig. 1). These results suggest that cDNA array analysis enables rapid and efficient identification of many relevant genes potentially related with DNA-PK activity.

With cDNA array, we found that most proteins that were related with DNA-PK activity are involved in regulation of cell cycle (Table I). However, approximately 99% of PBLs were in G0 phase, determined by flow cytometry (20). Our results demonstrate that individuals have different levels of expression of proteins involved in cell cycle and check-point regulation in PBLs although their PBLs are almost all in G0 phase.

We considered how the expression of the DNA-PK activity-related genes was cooperatively regulated. We paid attention to E2F1 as it had a strong correlation with the DNA-PK activity (Table I). The E2F transcription factor is regulated in a cell cycle-dependent manner and fluctuations in E2F activity programs of gene expression coupled closely with cell cycle position. The E2F family of transcription factors is composed of six members (E2F-1 to -6) that form DNA-binding heterodimers with members of the related DP family of co-activators (DP-1 to DP-3). In proliferating cells, E2F proteins activate transcription of several genes important for cell cycle progression (21). The genes transcriptionally regulated by E2F transcription factors include genes coding for cell cycle regulatory proteins: cyclin E, cyclin A, CDC2, CDC25, the CDK inhibitor p21 and proto-oncogenes such as Myc and Myb (22).

In contrast, in growth-inhibited cells as well as at initial stages of G1 progression, E2F proteins are targeted by the retinoblastoma susceptibility protein (pRB), p107 and p130 and are converted into transcriptional repressors of many genes that are positively regulated during the cell cycle. The expression of p130 had a strong negative correlation with

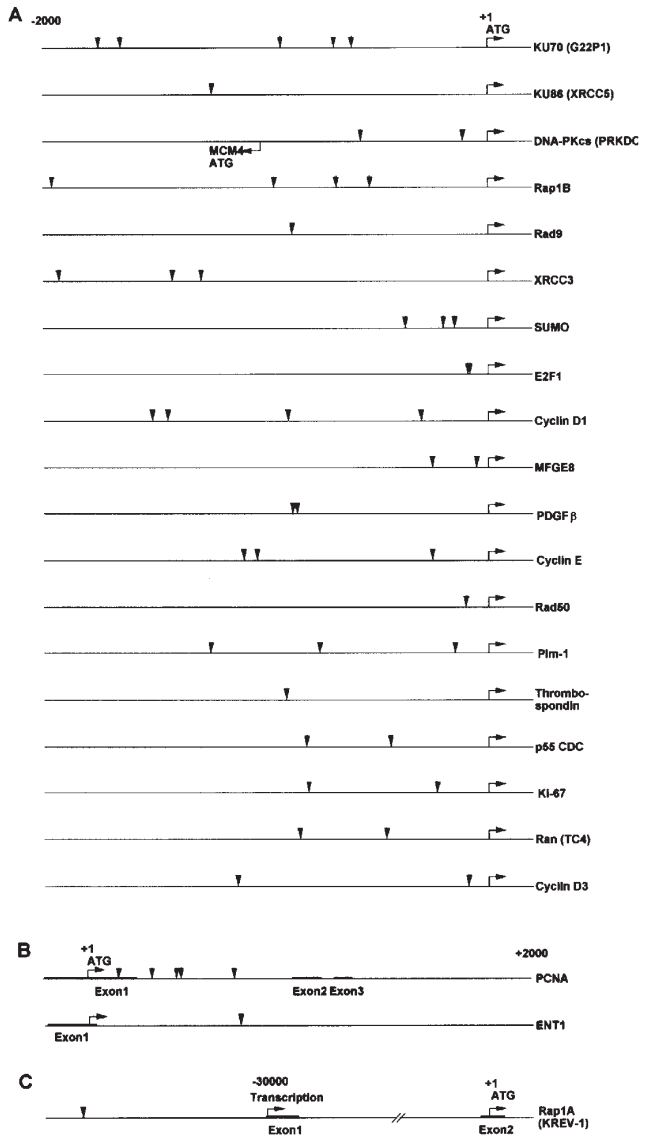

Figure 4. *In silico* promoter analyses. Potential E2F binding sites are highlighted with arrowheads. (A) 2 kb region upstream of the translation initiation site of each gene as indicated. In the case of DNA-PKcs, the translation initiation site of MCM4 is also indicated. (B) 2 kb region downstream of the translation initiation sites of PCNA and ENT1. (C) Region around the transcription and translation initiation sites of Rap1A.

the expression of E2F1 and DNA-PK activity (Table I and Figs. 1 and 5), suggesting that p130 might regulate the expression of genes that were related with DNA-PK activity via negative regulation of E2F function.

Although the levels of pRB are quite constant during the cell cycle and in quiescent cells, levels of p130 and p107 change dramatically depending upon the stage of the cell cycle. When cells progress through the G0/G1 transition and through mid G1, p130 is hyperphosphorylated, and dramatically down-regulated as cells move to S-phase (23,24). p130 levels are regulated by the rate of protein synthesis and/or by changes in protein stability linked to specific cell cycle stages. However, the mechanism of down regulation of p130 levels is not clearly elucidated (21).

We performed *in silico* promoter analyses of genes that were related with DNA-PK activity in order to investigate if there are E2F binding sites in promoter regions of these genes. E2F binding sites exist in promoter regions in genes with

Table II. Changes of gene expression after PHA activation.

Personal number Gene name[a]	1 Expression ratio[b]	2 Expression ratio[b]
PCNA (proliferating cell nuclear antigen)	76.9	93.7
Rap 1B	3.45	2.4
Rad9	1.89	13.2
XRCC3	8.23	23
Ku70	2.2	2.89
Ran GAP1 (Ubl1)	2.4	34.7
E2F1	85.5	73.7
Cyclin D1 (CYL1)	46.1	3.1
MFGE8 (milk fat globule-EGF factor 8 protein)	1.67	2.98
PDGF b	0.83	2.91
Cyclin E	0.3	0.19
hRAD50	42.6	17.4
Ku80	5.4	11.9
Rap 1A (KREV-1)	15.7	24.4
Pim-1	19.8	1.09
Thrombospondin 3	0.95	6.5
ENT1 (es transporter)	148	274
p55 CDC	50.8	3820
Connexin 32	11	2.81
PLC-γ1	1	5.81
Connexin 43	1.55	0.47
Ki-67	113	180
GAK (cyclin-G associated kinase)	1.22	6.18
ARF4	14.45	5.7
CAS (cellular apoptosis susceptibility)	3.27	359
Ran	3.7	14
GSTT1 (glutathione S-transferase T1)	10.5	5.74
Cyclin D3 (CYL3)	9.43	8.79
MMP7 (PUMP-1)	8.52	7.04
Integrin α6 (CD49F)	2.53	2.38
JAK1	3.05	0.99
GM2 activator protein	10	6.14
Granzyme B	7.73	9.03
Integrin ß1 (CD29)	8.83	2.79
Rb p130	0.652	0.243

[a]Genes are arranged in order of the highest relationship with DNA-PK activity. [b]Ratio of expression level after PHA stimulation to that before PHA stimulation.

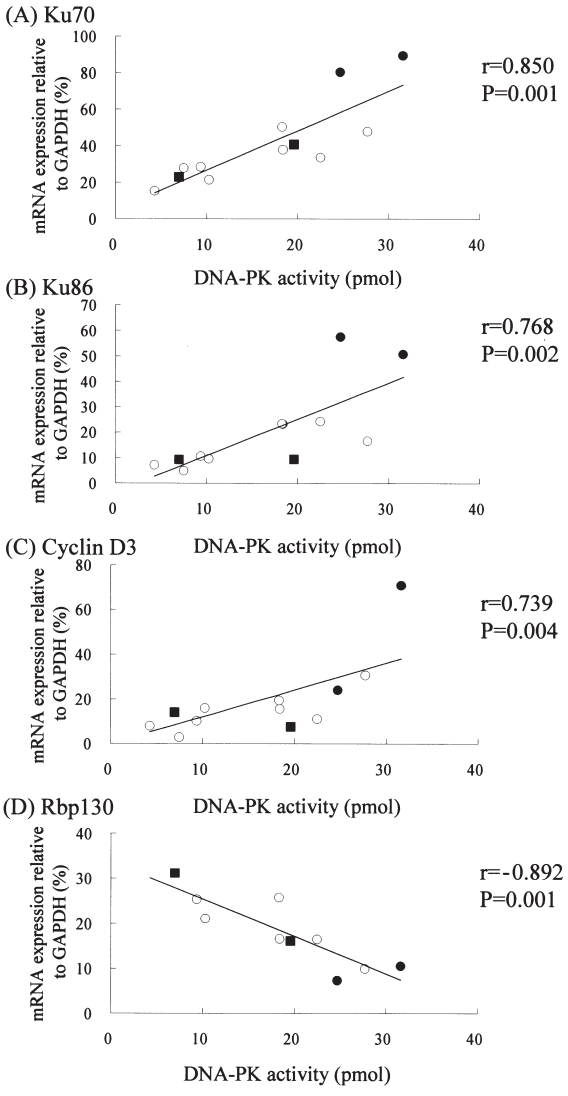

Figure 5. The relationship between DNA-PK activity and expression of genes after PHA stimulation. The expression level of these proteins is indicated by values relative to GAPDH. Closed squares indicate expression without PHA stimulation and closed circles expression after PHA stimulation. Open circles indicate the data of Table I.

close relationship to DNA-PK activity (Fig. 4), indicating that the E2F transcription factor may regulate the concerted expression of genes that are related with DNA-PK activity.

Next, we progressed cell cycle of PBLs with the mitogen PHA to stimulate the expression of cell cycle related genes. Then, we measured DNA-PK activity and analyzed gene expressions with cDNA array to ascertain the relationship between DNA-PK activity and expression of cell cycle related genes identified in G0 phase of PBLs. The activity of DNA-PK increased and expression of various genes including Ku70 and Ku86 was increased in PBLs after PHA stimulation (Table II and Fig. 5). These results agreed with those of Yaneva and Jhiang (25) examining expression of c-myc, Ku70, and Ku86 in PHA-stimulated PBLs with Northern blot and Western blot analyses. In that study, the level of mRNAs and protein of Ku70 and Ku86 were increased when the PBLs were stimulated to proliferate with PHA.

The expression of most genes in PHA-stimulated PBLs had a similar relationship with DNA-PK activity to that without PHA stimulation (Fig. 5). That is, the DNA-PK activity increased as mRNA of Ku70, Ku86, XRCC3, cyclin D1, SUMO, and E2F1 increased after PHA stimulation. The DNA-PK activity increased as mRNA of Rbp130 decreased. The expression of cyclin E decreased after PHA stimulation. The transcription of cyclin E is known to be E2F-dependent, but recently, several investigators have demonstrated the possible involvement of Sp1 transcription factor in cyclin E expression (26,27). Regulation of cyclin E expression by other than E2F may influence declined expression of cyclin E after PHA-stimulation.

It is asserted that in response to DNA damage, both cell cycle checkpoint mechanism (caretaker function) and DNA repair mechanism (gatekeeper function) act synergically and this cooperation is important for maintenance of genomic integrity and tumor suppression (28,29). Such check-point mechanisms arrest the cell cycle in response to DNA damage to allow the cell time to repair the DNA damage, or, if the damage is too extensive, they trigger apoptosis. However, the exact mechanism for the cooperation between check-point mechanism and DNA repair mechanism is not completely understood. Our results suggest that there may be net-working between the check-point and DNA repair molecules at gene expression level, which could be one of the mechanisms of cooperation between checkpoint and DNA repair mechanism.

In conclusion, we applied cDNA array technology to identify the expression of genes which are associated with DNA-PK activity by using PBLs with various DNA-PK activities. Most genes which were related with DNA-PK activity were cell cycle related. The E2F transcription factor that plays an important role in cell cycle regulation had a strong correlation with the DNA-PK activity. *In silico* promoter analyses showed E2F binding sites in promoter regions in genes with close relationship to DNA-PK activity. In order to examine the relationship among the E2F1 expression, the expression of genes related with DNA-PK acitivty, and DNA-PK activity, we activated PMLs by PHA to progress the cell cycle. After PHA activation of PML, the expression of E2F1 and DNA-PK activity increased. The expression of most genes in PHA-stimulated PBLs had a similar relationship with DNA-PK activity to that without PHA stimulation. These results indicate that the E2F transcription factor may regulate the concerted expression of genes related with DNA-PK activity.

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