Original Paper

Journal of Biomedical Science

J Biomed Sci 1998;5:101–110

Received: November 19, 1996
Accepted: June 16, 1997

Protein-Tyrosine Kinase and Protein-Serine/Threonine Kinase Expression in Human Gastric Cancer Cell Lines

Jyh-Shi Lin[a]
Chi-Wei Lu[a]
Chang-Jen Huang[b]
Peng-Fyn Wu[a]
Daniel Robinson[c]
Hsing-Jien Kung[c]
Chin-Wen Chi[e]
Chew-Wun Wu[d]
Wen-Kang Yang[a]
Jacqueline J.K. Whang-Peng[a]
Wen-chang Lin[a]

[a] Institute of Biomedical Sciences, Academia Sinica and Clinical Cancer Center, National Health Research Institutes, and
[b] Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan, ROC;
[c] Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA;
Departments of
[d] Surgery and
[e] Medical Research, Veterans General Hospital-Taipei and School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC

Key Words
Protein tyrosine kinase
Protein serine/threonine kinase
RT-PCR
Gastric cancer

Abstract

Protein kinases play key roles in cellular functions. They are involved in many cellular functions including; signal transduction, cell cycle regulation, cell division, and cell differentiation. Alterations of protein kinase by gene amplification, mutation or viral factors often induce tumor formation and tumor progression toward malignancy. The identification and cloning of kinase genes can provide a better understanding of the mechanisms of tumorigenesis as well as diagnostic tools for tumor staging. In this study, we have used degenerated polymerase-chain-reaction primers according to the consensus catalytic domain motifs to amplify protein kinase genes (protein-tyrosine kinase, PTK, and protein-serine/threonine kinase, PSK) from human stomach cancer cells. Following amplification, the protein kinase molecules expressed in the gastric cancer cells were cloned into plasmid vectors for cloning and sequencing. Sequence analysis of polymerase-chain-reaction products resulted in the identification of 25 protein kinases, including two novel ones. Expression of several relevant PTK/PSK genes in gastric cancer cells and tissues was further substantiated by RT-PCR using gene-specific primers. The identification of protein kinases expressed or activated in the gastric cancer cells provide the framework to understand the oncogenic process of stomach cancer.

Introduction

There are two major classes of protein kinases, protein-tyrosine kinase (PTK) and protein-serine/threonine kinase (PSK). These kinases share common sequence motifs in their catalytic domain [6]. They play extremely important roles in the cell growth, cell cycle regulation and development, especially in the signal transduction pathways [4, 8].

While the abundance of PTK genes accounts for less than 10% of all cellular kinases, nearly all of them are involved in growth signaling [9]. Many PTKs have been shown to be oncogenic once they have lost their biological regulation either by gene amplification, somatic mutation

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

S. Karger AG, Basel
1021–7770/98/0052–0101$15.00/0
This article is also accessible online at:
http://BioMedNet.com/karger

Wen-chang Lin, PhD
Institute of Biomedical Sciences
Academia Sinica
Taipei 115, Taiwan (ROC)
Fax +886 2 2873 3664, E-Mail WenLin@ibms.sinica.edu.tw

or viral activation [2, 3, 22, 24]. In human gastric cancers, the amplification and over-expression of protein-tyrosine kinases have been implicated in progression processes. C-erbB-2/neu, a member of EGFR family, is found to be frequently amplified in human cancers, including gastric cancers [15, 29]. C-met proto-oncogene is found to be associated with gastric cancer progression [16]. PSKs are important intracellular regulators, which receive cellular signals from tyrosine kinases and transmit them into the nucleus. Inside the nucleus, they are responsible for phosphorylating transcription factors, cell-cycle regulators, etc. [4]. Because of their vital roles in cellular processes, many PTKs and PSKs are ubiquitously expressed and tightly regulated. They are, however, kinases whose expression are cell type specific or tumor specific. Identifying the latter type should significantly enhance our understanding of the differentiation and oncogenesis process.

To date, a large number of protein kinases have been identified [10]. Because of the complexity and cross-talk involved in the kinase-kinase, kinases-substrate interactions, it is important to identify a general PTK/PSK expression profile in a particular cancer type. This should provide important leads to the growth and oncogenic signals involved in that cancer cell type. Subsequently, one can focus more on specific relevant kinase genes among PTK/PSK genes identified. Because of the importance of protein kinase genes for many cellular functions, the catalytic domain of protein kinases is conserved within the protein kinase families during evolution and invariable motifs can be identified [7]. Oligonucleotide probes corresponding to the highly conserved kinase motifs have been used to screen cDNA libraries for new PTK/PSK genes [5], and recently degenerate PCR primers from conserved kinase motifs for amplification and identification of kinase molecules have also been designed [26]. In order to establish such a general kinase expression profile in human cancer cells, an improved reverse transcriptase-polymerase chain reaction (RT-PCR) method has been developed to identify a variety of kinase genes in a single screen [19].

Adenocarcinoma of stomach is one of the most important malignancies in Taiwan [27]. It is the third leading cause of death in cancer patients in Taiwan. In this report, we have used degenerate primers from conserved regions of kinase domain in RT-PCR analysis. In addition to DVW primer specific for PTK profiling previously described [19], we have included a newly designed D(I/L/M)W primer for PSK profiling. From one gastric cancer cell line analyzed, 25 kinase genes were identified including twelve PTK genes, seven PSK genes, four dual kinases and possibly two new genes. We have also verified the expression of several relevant PTK/PSK genes in four human gastric cancer cell lines and one pair of surgical specimens (normal gastric mucosa and gastric cancer tissues) by a gene-specific RT-PCR method.

Materials and Methods

Gastric Cancer Cell Lines and Surgical Specimen

Four gastric cancer cell lines were used in this study, including HR [21], AGS [28], KATO III [28] and SC-M1 [14]. These cells were cultured in RPMI 1640 or DMEM culture medium supplemented with 10% fetal calf serum, 2 m*M* glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2/95% air at 37°C. Gastric cancer tissue (TL1) and its corresponding normal gastric mucosa tissue (NL1) were obtained from Dr. Chew-Wun Wu at Veteran General Hospital, Taipei. Informed consent was obtained from the patient. The specimens were immediately frozen in liquid nitrogen after resection and used for RNA isolation.

RNA Purification from Gastric Cancer Cell Lines and Surgical Specimen

The direct quanidine isothiocyanate lysis and cecium chloride gradient separation method was used for exacting total RNA from gastric cancer tissues [17]. Briefly, cultured gastric cancer cells or gastric cancer tissues were lyzed in a lysis solution containing 4 *M* quanidine isothiocyanate. The lysate was over-laid onto a layer of 5.7 *M* cecium chloride and spun at 90,000 rpm for 5.5 h on a Beckman TLX-100 ultracentrifuge. The RNA pellets were washed several times with 70% ethanol, dried and resuspended with RNase-free water. PolyA+ mRNA of HR cells was further isolated with a PolyAtract mRNA isolation system from Promega (Wisc., USA).

RT-PCR Amplification and Sequence Analysis of PTK/PSK Genes

For PTK/PSK profiling of HR cells, reverse transcription was carried out with 2 μg polyA selected mRNA, oligo $(dT)_{15}$ and MuLV reverse transcriptase from Promega. A degenerate kinase specific primer W(S/A)(FL/YC)G, R2, was used in place of oligo $(dT)_{15}$ in subsequent reverse-transcription experiments. This primer overlaps with the R1 and R4 primer, which enhances the sensitivity by enriching the kinase-related transcripts. The quality of RT products was examined by agarose gel electrophoresis and by PCR reaction with β-actin-specific primers.

The PCR primers are derived from the conserved motifs DFG and D(V/I/L/M)W of kinase catalytic domain VII and IX. DVW motif is primarily for PTK genes as previously described [19] whereas D(I/L/M)W motif is for PSK genes. Several pairs of degenerated PCR primers were designed from the following amino acid sequences:

For the 5′ primers (F1, F2, F3)

F1: 5′-(*Bam*HI)-K[V/I][S/C]DFG-3′
F2: 5′-(*Bam*HI)-K[V/I]GDFG-3′
F3: 5′-(*Bam*HI)-K[V/I][A/T]DFG-3′

For the 3′ primer (R1, R4)

R1: 5′-DVW[S/A][F/Y]G-[*Eco*RI]-3′
R4: 5′-D[I/L/M]W[S/A][F/Y]G-[*Pst*I]-3′

The PCR reactions were conducted at 42 °C annealing temperature for 5 cycles and then at 55 °C for 25 cycles with an Accugen 9600 PCR thermocycler and Takara Taq polymerase. The final PCR products were analyzed in 8% polyacrylamide gels. The 150 to 170-bp bands were eluted from gels and purified with a nucleartrap kit. Following ligation with T-vector plasmid from Promega, the resulting recombinants were screened for the proper 150-170 inserts by *Bam*HI/*Eco*RI or *Bam*HI/*Pst*I digestion of plasmid DNA.

Following the identification of recombinants, plasmid DNA was isolated with Qiaprep spin miniprep kit from Qiagen Inc. (Calif., USA). Sequence analysis was done with automatic sequencing service in Institute of Biomedical Sciences equipped with an Applied Biosciences autosequencer. The sequences were then analyzed and translated into amino acid sequences with a Macintosh SeqApp program for identification of the DFG and D(V/I/L/M)W motif. In order to compare with known PTK genes, computer comparison with GeneBank, EMBL data bases were performed with the BLAST program.

RT-PCR Expression Analysis of Selected Kinases

Total RNA samples from various gastric cancer cell lines and tissues were used in reverse transcription reactions with oligo $(dT)_{15}$ primers as previously described. The resulting cDNA were subjected to PCR reaction by using gene-specific primers. The PCR was conducted in 25 µl reaction containing 200 µ*M* each dNTP, 1.25 m*M* $MgCl_2$, and 800 n*M* each of the specific primer for 35 cycles at 58 °C annealing temperature for 30 s, 72 °C for 30 s, and 94 °C for 30 s. The primer sets for the kinases studied are as follows:

	Sense	Antisense
GAPDH	TGGTATCGTGGAAGGACTCA	AGTGGGTGTCGCTGTTGAAG
KAI	CATCGTGACTGAGCT	GAGCTCGATGATGGC
DPC4	CTGTTGATGGATACGTGG	TGAACAGCATCTCCAG
YES	GTGATTGGGATGAGATAAG	TGATTGCTACTTTCGTGG
TYK2	GGCAAGATGGATGACGAG	TGGGTTGGCTCATAGGTC
FLG	GGTCGGTCATCGTCTACA	CTGAGATCAGGTCTGACA
NYK/MER	GCAGCATTCAGGTCAAGG	GCTTAGTCGGAGGCTTCA
NEK2	GACTCAGTTGACTCTGG	GAGAGCTCGCTCTCGCT
PLK	GTGCTTGGCTGCCAGTA	CTGTCTGAAGCATCTTC
MEK2	CAGCATTTGCATGGA	CGACTGCACCGAGTA
MKK4	CAGTGTTAGTGCTGGTC	ATGTGTCTCGTTGGCTG
BEK	CTGGTGTCAGAGATGGAG	CATCCACTTGACTGGAAG
K-SAM (TK)	AGAGTATACACTCATCAGAGTGATGTC	
K-SAM (C1)	CAAGTGGAGACAACAAGCTCT	
K-SAM (C3)	GTTTCTCAATGAAGCCATAAACTTTCAGAT	

The expected sizes of PCR products, are: 371 bp for GAPDH, 373 bp for KAI, 224 bp for DPC4, 318 bp for YES, 710 bp for TYK2, 417 bp for FLG, 285 bp for NYK/MER, 583 bp for NEK2, 368 bp for PLK, 316 bp for MEK2, 268 bp for MKK4, 420 bp for BEK, 532 bp for K-SAM (TK-C1) and 298 bp for K-SAM (TK-C3). The final products were analyzed in 2% agarose gel, visualized by ethidium bromide staining and recorded with Alpha Innotech Co. IS-500 gel documentation system.

Results

In order to examine the PTK/PSK expression profile and their significance in human gastric cancer oncogenesis and progression, we selected a human gastric cancer cell line, HR, for our present study. The PTK/PSK profiles established here represents PTK/PSK expression in homogeneous gastric cancer population and is useful in studying the biological significance of PTK/PSK on the growth and progression of gastric cancer cells. This profile can serve as a baseline for gastric cancer PTK/PSK expression. It can be used to compare the PTK/PSK profile established from clinical samples which contain heterogeneous cell populations. The present study, however, is by no means exhaustive but paves the way to build up a comprehensive kinase expression profiles in this cancer type. In this report, we have included a new degenerate primer (R4) for PSK profiling, in addition to the PTK specific primer (R1) described earlier [19].

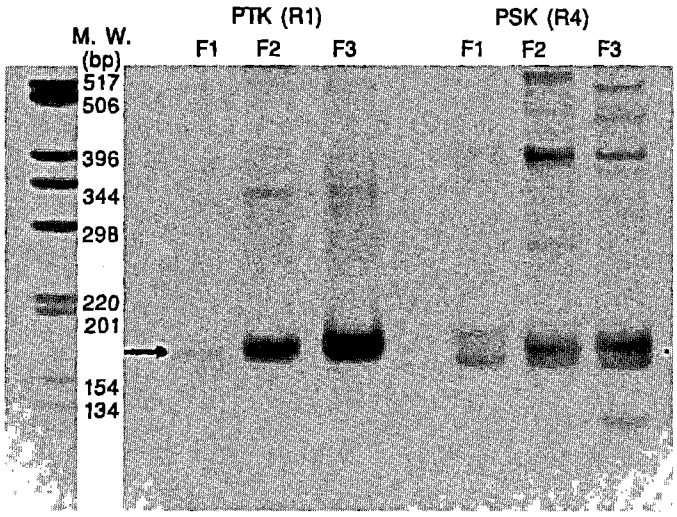

Fig. 1. 165–170 bp PTK/PSK RT-PCR products separated on an 8% polyacrylamide gel. PolyA selected mRNA obtained from a gastric cancer cell line, HR, was used. RT-PCR using F1, F2, F3, R1 and R4 combination was performed as described in 'Materials and Methods'.

When we tested these degenerate PTK/PSK primers, ~ 150–170 bp RT-PCR products could be detected with oligo $(dT)_{15}$ primed mRNA from the HR gastric cancer cell line, as shown in figure 1 (indicated by an arrow). Because of degeneracy, three forward primers were designed, and two reverse primers were used. R1 is specific for DVW motif of PTK genes, while R4 is specific for D(I/L/M)W motif of PSK. The F1-R1 and F1-R4 combination yielded the least PCR products; while the F3-R1 and F3-R4 reaction gave the most PCR products. However, some nonspecific amplified products were also observed. This may result from the low annealing temperature used in the PCR reaction. In subsequent experiments, we observed a similar RT-PCR pattern when we used the

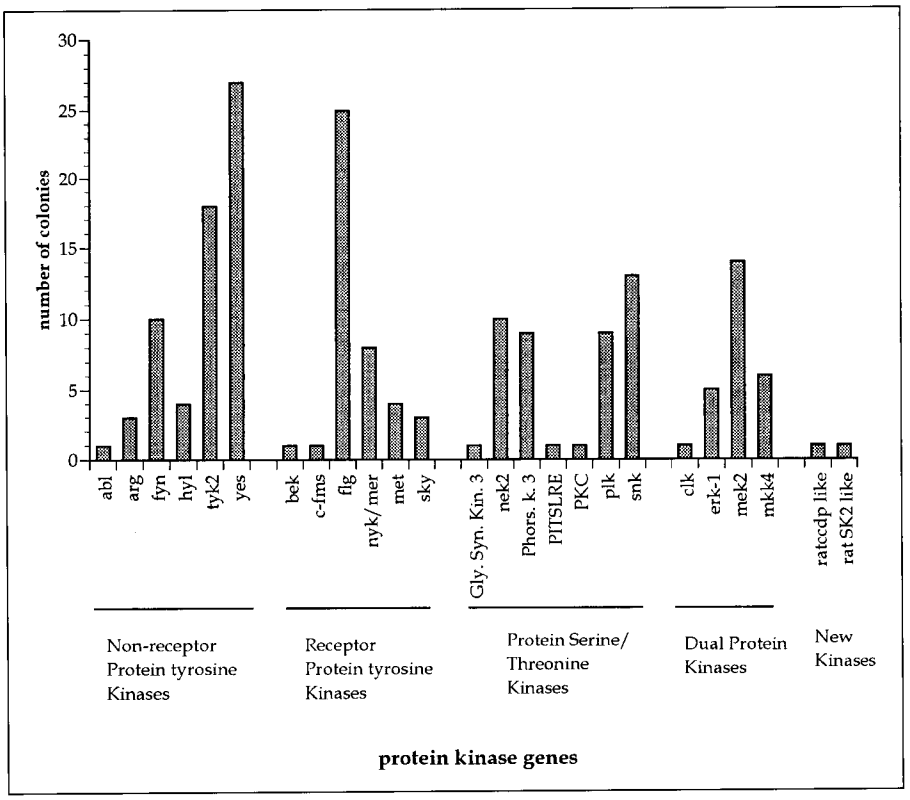

Fig. 2. Profiles of PTK/PSK genes expressed in a human gastric cancer cell line, HR.

kinase related primer, R2, to initiate the reverse transcription reaction. In both cases, we purified the 170-bp PCR products for subsequent analyses.

The amplified products were then subcloned into a plasmid vector for DNA sequencing. We sequenced about 200 positive clones with a 170-bp insert. The sequence data was entered via a Macintosh computer and analyzed with various data bases as described in 'Materials and Methods'. Using this approach, we recognized protein kinase gene sequences from 89 PTK clones (60 clones with oligo $(dT)_{15}$ and 29 clones with R2 primed) and from 88 PSK clones (53 clones from oligo $(dT)_{15}$ and 35 clones from R2). The others were products of PCR errors or unidentifiable sequences. The profile of PTK/PSK genes is illustrated in figure 2, and a total of 25 different PTK/PSK genes were identified. We classified them into five major groups of kinase families, including (a) PTK group; (b) AGC group; (c) CaMK group; (d) CMGC group, and (e) other kinase group according to a recent review by Hanks and Hunter [6].

The PTK genes include nonreceptor and receptor type PTK genes. In nonreceptor PTK genes identified here, two belong to the abl family (abl, arg); two belong to the src family (fyn, yes); one (tyk2) is in the jak family; one (hyl) is in the csk family. Among receptor PTKs, there are two members (flg, bek) of the fibroblast growth factor receptor family; two members (nyk/mer, sky) of the ufo/axl family. C-fms is the colony stimulating factor receptor, and met is the hepatocyte growth factor receptor.

In PSK and dual kinase genes (tyrosine kinases and serine/threonine kinases) identified here, PKC belongs to the AGC group of the kinase family; phosphorylase kinase is in the CaMK group; Gly. Syn. Kinase 3, PITSLRE, clk and erk-1 belong to their respective families within the CMGC group; while nek2 is in the NIMA family, plk and snk are the members of plk family; mek2 and mkk4 are in the MEK family.

Two possible new kinases were also identified:

ratccdp like DFGVSNEFKGSDALLSNTVGTPAFMAPESLSETRKIFSGKALDVW

–73% amino acid homology and 71% nucleotide homology with ratccdp gene.

rat SK2 like DFGVSAKNTRTIQRRDSFIGTPYWMAPEVVMCETSKDRPYDYKADVW

–100% amino acid homology with rat SK2 gene (fragment).

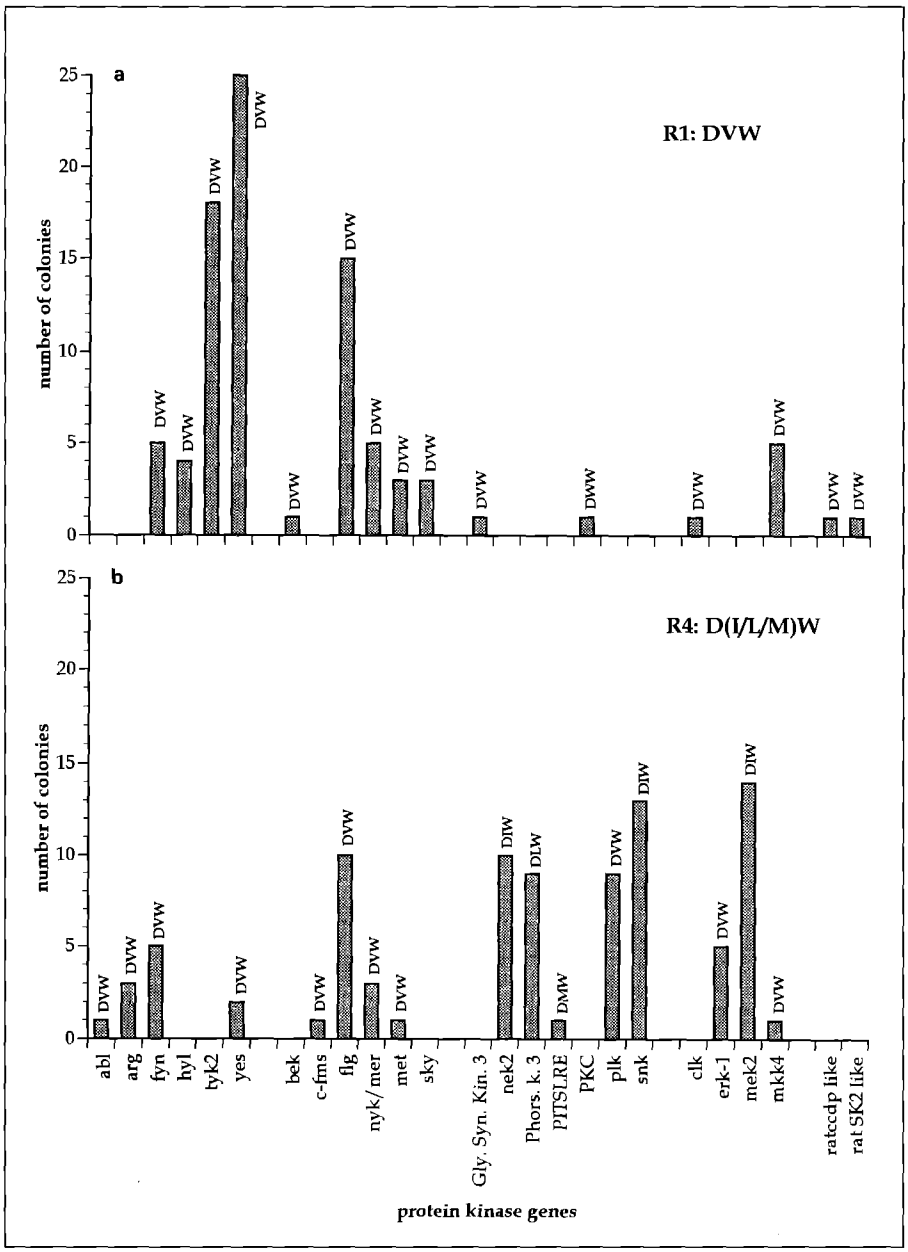

Fig. 3. a Profiles of PTK genes expressed in a human gastric cancer cell line, HR. The subdomain IX motif of each gene is indicated. **b** Profiles of PSK genes expressed in a human gastric cancer cell line, HR. The subdomain IX motif of each gene is listed on top of each bar.

Their identities are presently under investigation. The ratccdp like gene is also identified during PTK profiling of human gastric cancer tissues [Lin et al., submitted]. The rat SK2 like gene is also identified as in the PTK profile of human colon cancer tissues [Chen and Lin, unpubl. observation].

When we examined the distribution of PTK and PSK genes (as shown in fig. 3), the specificity of DVW (R1) and D(I/L/M)W (R4) reverse primers are clearly demonstrated. Almost all DVW (R1) reverse primer generated clones contain DVW motif in their kinase subdomain, except for PKC (fig. 3, top panel). The two possible new kinases should also contain DVW motif according to their closest related genes. The D(I/L/M)W (R4) reverse primer is less specific than DVW (R1) primer. In addition to D(I/L/M)W motif containing PSK genes, many DVW motif PTK genes can also be amplified by this primer (fig. 3, lower panel). This could result from the higher degeneracy of the D(I/L/M)W primer than the DVW primer. We also compared the difference between oligo $(dT)_{15}$ primed

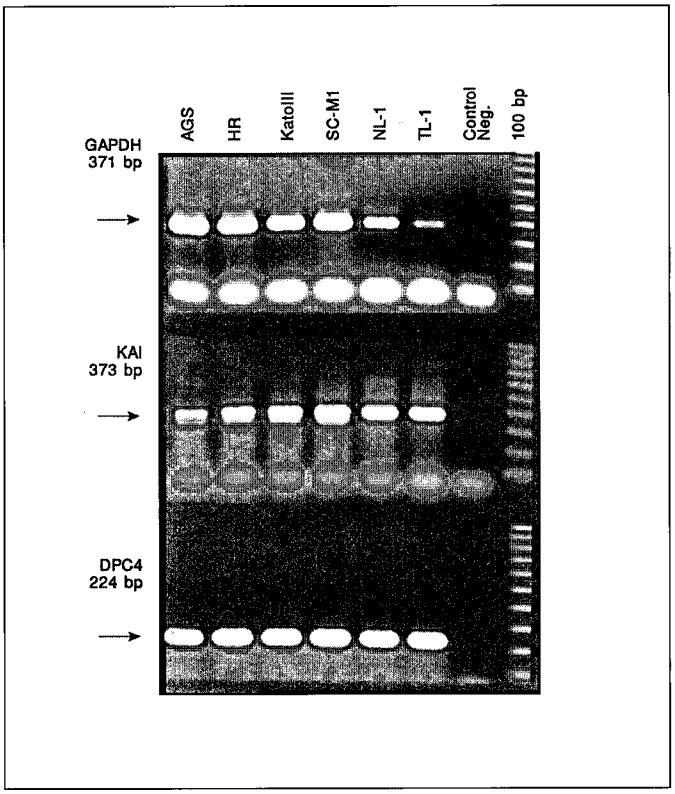

Fig. 4. Expression analysis of GAPDH, DPC4, and KAI genes in four gastric cancer cell lines (AGS, HR, KATO-III and SC-M1) and one pair of clinical specimen (including normal gastric mucosa, NL1, and gastric cancer tissue, TL1). The detailed procedures and RT-PCR primers used are described in 'Materials and Methods'. The final products were analyzed in 2% agarose gel.

cDNA and kinase-specific R2-primed cDNA. No significant difference could be observed with oligo $(dT)_{15}$ or R2-primed cDNA (data not shown).

Because of the limited number of clones analyzed thus far, we used this profile only for identification purpose. No attempt was made to strictly correlate the expression level and the number of colonies identified. Nevertheless, several kinases which could play important biological roles in this gastric cancer cell line were identified because of their higher expression frequency in the profile. These PTK/PSK genes were selected for further examination by RT-PCR on other gastric cancer cell lines and on one pair of clinical sample (normal gastric mucosa and gastric cancer tissues). Most of these PTK/PSK genes have not been investigated in human gastric cancer cells. They include: tyk2, yes, flg, nyk/mer, plk, nek2, mek2, mkk4. A housekeeping gene (GAPDH), and two tumor suppressor genes (DPC4, KAI) were used as RT-PCR controls (fig. 4). Specific and expected sized bands were identified. No alterations in the two recently discovered tumor suppressor genes (DPC4 and KAI) were found in gastric cancer cell lines. As shown in figure 5a–d, four gastric cancer cell lines (AGS, HR, KATO III, SC-M1) as well as one pair of surgical specimen (including normal gastric mucosa, NL1, and gastric cancer tissue, TL1), expressed all the kinase genes examined. This confirms the initial RT-PCR results. Our approach is only semiquantitative, however significant variations of the expression of some of the kinases were noted. (For instance, the tyk2 PTK is expressed much less in the SC-M1 cells as shown in figure 5b.)

One FGFR family PTK gene, bek, could have important roles in gastric cancer cell growth and oncogenesis. Since we have identified the FGFR1 (flg), FGFR2 (bek/k-sam) in this profile analysis, we decided to further investigate the bek/k-sam gene. The k-sam gene of FGFR family PTK is identified from gastric cancer cells and its expression has been correlated with gastric cancer progression [11, 13]. K-sam represents an alternatively spliced form of bek. Using the primer sets described in Itoh et al. [12], an alternatively spliced form of k-sam (C3) could be detected in Kato III gastric cancer cell, in addition to the frequently observed k-sam (C1) transcript. This alternatively transcribed C3 message represents a C-terminal truncated form of FGFR protein, which lacks the tyrosine 769 residue. As shown in figure 6, all cell lines express the bek transcripts with SC-M1 expressing the least. Likewise, k-sam C1 transcript is present in all cell lines with the lowest expression in SC-M1. Only the KATO III expresses the C3 transcript [12].

This type of approach could be useful in understanding the basic PTK/PSK profiles of various gastric cancer cell lines and studying specific kinase genes in the progression of gastric cancer cells. The identification of PTK/PSK expressed in gastric cancer cells may also provide diagnostic evaluation of stomach cancers, as well as elucidate the basic molecular biology of the protein-tyrosine kinases in gastric cancer oncogenesis.

Discussion

Incidence of stomach cancer is the third highest type among cancer patients in Taiwan. Because of the importance of PTK/PSK genes in signal transductions and oncogenesis, we examined the profiles of PTK/PSK gene expression in a human gastric cancer cell line. Following RT-PCR amplification, the PTK/PSK expressed in gas-

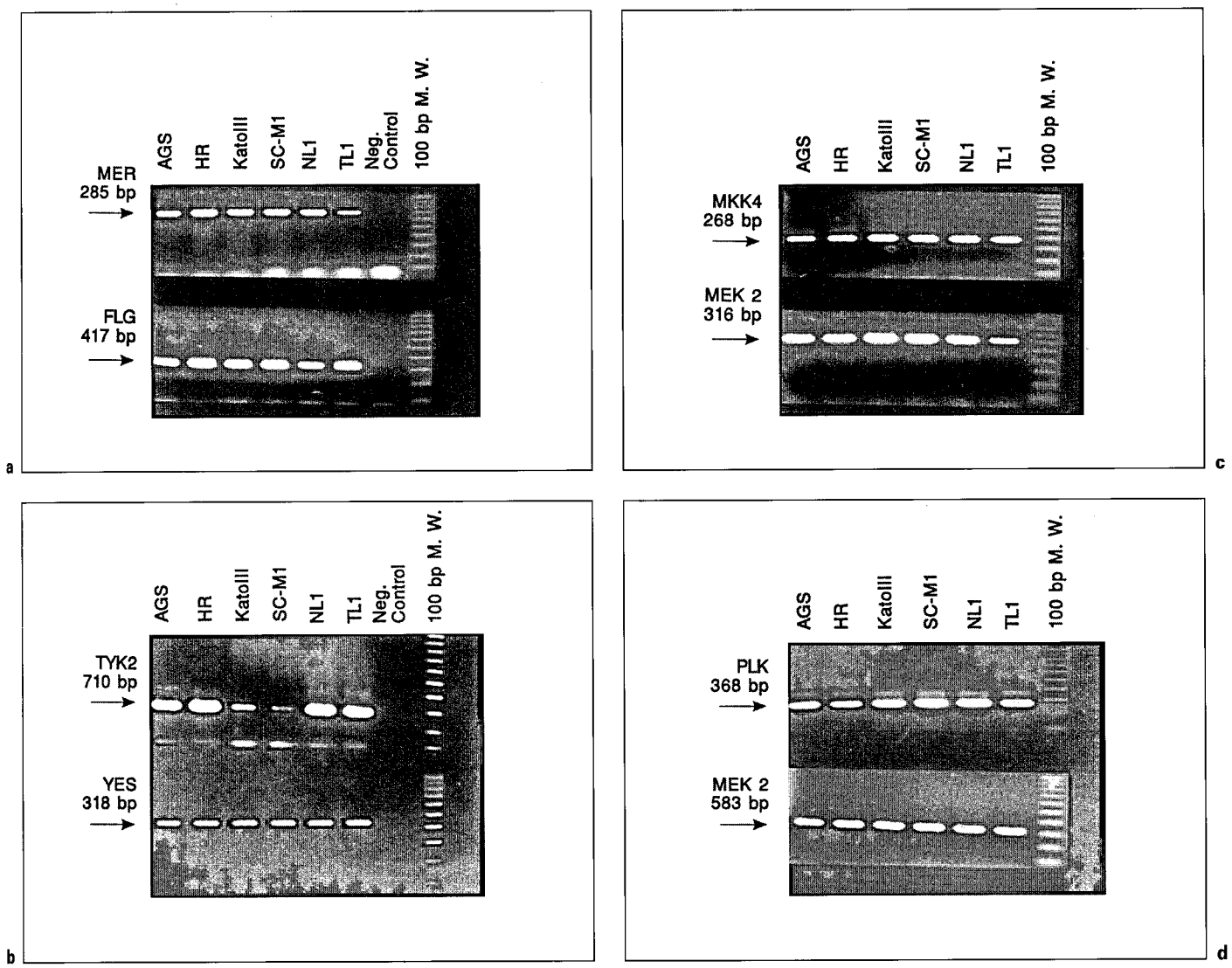

Fig. 5. Expression analysis of selected kinases in four gastric cancer cell lines (AGS, HR, KATO-III and SC-M1) and one pair of clinical specimen (including normal gastric mucosa, NL1, and gastric cancer tissue, TL1). RT-PCR was used to study the general expression pattern of two selected kinases from four major subgroups identified in our profile analysis, tyk2 and yes from nonreceptor PTK (**a**); flg and nyk/mer from receptor PTK (**b**); mek2 and plk from PSK group (**c**); mek2 and mkk4 from dual kinases (**d**). The detailed procedures and RT-PCR primers used are described in 'Materials and Methods'. The final products were analyzed in 2% agarose gel.

tric cancer cells were cloned and their sequences analyzed. Our efforts have identified 25 different protein kinases in human gastric cancer cells. Our primary goal was first to identify a variety of kinases present in gastric cancer cells with this approach and then to examine the expression of individual kinases identified from the profile by a more quantitative means. Many PTK/PSK genes reported here have been shown previously to be oncogenes or to have transforming activity, like abl, arg, fyn, yes, bek, c-fms, met, nyk/mer, sky, etc. Their significance in gastric cancer oncogenesis process remains to be investigated. Two of the PTK genes identified (c-met and bek/k-sam) have been demonstrated to be involved in the progression of human gastric cancers [11, 12, 15]. This approach would be useful in studying kinase related oncogenes expressed in human gastric cancer cells.

Similar PCR approaches have been used for isolating novel PTK genes by several groups. The subdomains VI

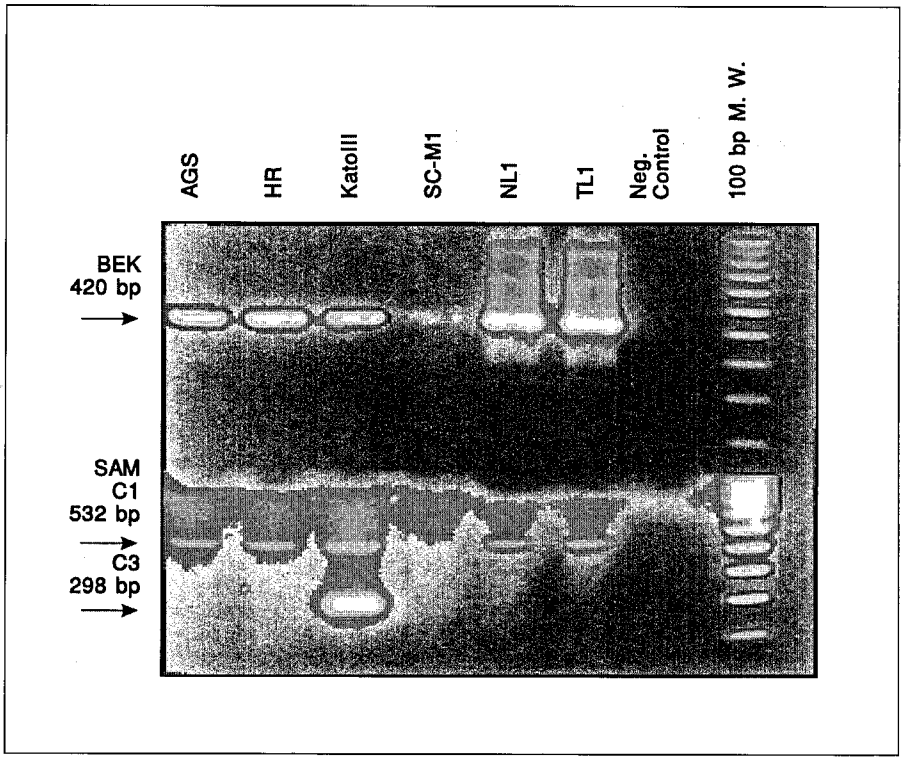

Fig. 6. Expression analysis of bek/k-sam PTK gene in four gastric cancer cell lines (AGS, HR, KATO-III and SC-M1) and one pair of clinical specimens (including normal gastric mucosa, NL1, and gastric cancer tissue, TL1). The detailed procedures and RT-PCR primers used are described in 'Materials and Methods'. The final products were analyzed in 2% agarose gel.

(xHRD.Kxx) and IX (DVWxxG) have been frequently used, the combination of which has produced a PCR product at around 210 bp. With degeneracy of 32 and 8 of IHRDL and DVWSFG primers, Wilks et al. identified 6 different PTK genes among 200 clones analyzed and over 90% of inserts were PTK related [25, 26]; Mossie et al. [1, 18] identified 26 PTK genes in 250 PCR clones analyzed with HRDLAA and D(V/M)WS(F/Y)G primers; Takahashi et al. [23] used the HRDLAAR and DVWSFGV primers and identified 24 PTK genes in 37 clones. In a detailed study by Schultz et al. [20], they used 11 degenerated primers covering subdomains V to IX to isolated PTK/PSK genes. They selected four forward primers (one for YLYMEYC at subdomain V, one for HRDLKP at domain VI and two for K(I/L)(A/C/G)DFG at domain VII), and three reverse primers (DVW(S/A)(C/L)G(V/I); D(I/M)WS(I/V)G; W(S/A)(I/V)GCI). A total of 41 PTK/PSK genes was identified from 316 clones. The success rate of identifying PTK/PSK-related genes ranged from 33 to 90%. In general, higher degeneracy can produce a more diverse array of kinase genes, but the specificities of priming are compromised. Thus, the efficiency of degenerate PCR amplification is influenced less by the primer length but is greatly affected by primer degeneracy, with lower degeneracy being most favorable [20]. Shorter primers also work more effectively at higher annealing temperatures. Thus, the specificity of PCR primers is enhanced.

In order to increase the varieties of PTK/PSK genes identified without sacrificing the PCR amplification efficacy, we designed three forward PCR primers corresponding to the lower degeneracy of 512, 256 and 512, and two reverse primers at the degeneracy of 1024 and 2048, in place of one primer with very high degeneracy. By this approach, the incubation temperature of our PCR reactions can be raised from 37 to 42°C during the first five cycles and 55°C at the following 25 cycles. This certainly increased the specificity in identifying PTK/PSK genes. In our study here, more than 95% of the sequences identified were kinase related genes. The number of kinases identified totalled 25 PTK/PSK genes for this gastric cancer cell line. In another study, we have identified more than 30 PTK genes with F1, F2, F3 and R1 combination from one pair of clinical specimen [unpubl. observations]. In addition, we selected the subdomain VII and IX combination. The relative homogeneous size distribution between DFG and D(V/I/L/M)W motifs makes the purification of the kinase band possible, and the 170 bp

overall size can be displayed in a single sequencing gel, making the comparison of different clones easier.

Bek/k-sam gene is generated from FGFR 2 gene by different RNA splicing mechanism. K-sam (C1) transcript is identical with KGFR (keratinocyte growth factor receptor), while k-sam (C3) is a truncated form of k-sam (C1). bek is different from k-sam genes in the second half of the third Ig-like domain [12]. Our study implicates the expression of bek/k-sam messages in almost all gastric cancer cell lines examined. Only the SC-M1 cells express low amounts of bek/k-sam mRNA. Kato-III is the only cell line which expressed a high level of the k-sam (C3) transcript. Its significance needs to be further investigated. Although this truncated C3 transcript has been shown to be transforming [12], Kato-III cells are nontumorigenic in nude mice. On the other hand, HR cells were highly tumorigenic in nude mice, which only express the k-sam (C1) message. It will be interesting to transduce k-sam (C1) and k-sam (C3) into the low-producing SC-M1 cells and then examine the phenotypic changes (like tumorigenicity, etc.) in these clones. The biological roles of the FGFR family in gastric cancer cells need to be further investigated.

PTK genes have been extensively studied and have contributed to significant findings regarding critical cellular signal pathways have been made [10]. Recently, PSK genes have been demonstrated to function in conjunction with PTK genes to form signaling circuits in cells. The application of R4 primer to identify PSK in this approach provides an opportunity to study signal transduction and uncover new PSK genes. We have used this method and identified eight new PSK genes in human natural killer cells [unpubl. observation]. The R2 primer overlaps with the R1 and R4 primer, which could enhance the sensitivity by enriching the kinase-related transcripts, in cases where kinase domain is far removed from the polyA sequence.

This report provides a new way to examine the tumor cell staging with different kinase gene expression. The data base established here gives clues to the involvement of specific PTK/PSK in the gastric cancer progression. The possible new or tissue-specific protein-tyrosine kinase genes identified in this study (rat SK2 like PTK and ratccdp like PTK) could also lead to new insights into the signal transduction pathway and cell cycle studies.

Acknowledgments

This work was supported in part by grant from the National Sciences Council (NSC-85-2331-B-001-004) and in part by the Department of Health, Executive Yuan, Taiwan, Republic of China.

References

1 Alves F, Vogel W, Mossie K, Millauer B, Hofler H, Ullrich A. Distinct structural characteristics of discoidin I subfamily receptor tyrosine kinases and complementary expression in human cancer. Oncogene 10:609–618;1995.

2 Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S. Oncogenes and signal transduction. Cell 64: 281–302;1991.

3 Chang CM, Shu HK, Kung HJ. Disease specificity of kinase domains: The src-encoded catalytic domain converts erbB into a sarcoma oncogene. Proc Natl Acad Sci USA 92:3928–3932;1995.

4 Edelman AM, Blumenthal DK, Krebs EG. Protein serine/threonine kinases. Annu Rev Biochem 56:567–613;1987.

5 Hanks SK. Homology probing: identification of cDNA clones encoding members of the protein-serine kinase family. Proc Natl Acad Sci USA 84:388–392;1987.

6 Hanks SK, Hunter T. The eukaryotic protein kinase superfamily. In: Hardie G, Hanks S, eds. The Protein Kinase Facts Book. San Diego, Academic Press, I:7–47;1995.

7 Hanks SK, Quinn AM, Hunter T. The protein kinase family conserved features and deduced phylogeny of the catalytic domains. Science 241:42–52;1988.

8 Hunter A, Cooper JA. Protein-tyrosine kinases. Annu Rev Biochem 54:897–930;1985.

9 Hunter T. A thousand and one protein kinases. Cell 50:823–829;1987.

10 Hunter T. 1001 protein kinases redux-towards 2000. Semin Cell Biol 5:367–376;1994.

11 Ishii H, Yoshida T, Oh H, Yoshida S, Terada M. A truncated K-k-sam product lacking the distal carboxyl-terminal portion provides a reduced level of autophosphorylation and greater resistance against induction of differentiation. Mol Cell Biol 15:3664–3671;1995.

12 Itoh H, Hattori Y, Sakamoto H, Ishii H, Kishi T, Sasaki H, Yoshida T, Koono M, Sugimura T, Terada M. Preferential alternative splicing in cancer generates a K-sam messenger RNA with higher transforming activity. Cancer Res 54:3237–3241;1994.

13 Iwase T, Tanaka M, Suzuki M, Naito Y, Sugimura H, Kino I. Identification of protein-tyrosine kinase genes preferentially expressed in embryo stomach and gastric cancer. Biochem Biophys Res Commun 194:698–705;1993.

14 Jin LD, Meng CL, Han SH, Ding MJ, Chang TM, Chan TK, Shen KL. A study on production of monoclonal antibodies using SC-M1 cells as immunogen. Med Sci 8:17–25;1987.

15 Kameda T, Yasui W, Yoshida K, Tsujino T, Nakayama H, Ito M, Ito H, Tahara E. Expression of ERBB2 in human gastric carcinomas: Relationship between p185ERBB2 expression and the gene amplification. Cancer Res 50: 8002–8009;1990.

16 Kuniyasu H, Xasui W, Kitadai Y, Yokozaki H, Ito H, Tahara E. Frequent amplification of c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun 189:227–232; 1992.

17 Maniatis T, Fritsch E, Sambrook J. Molecular Cloning: A Laboratory Manual. ed 1. Cold Spring Harbor, Cold Spring Harbor Laboratory, 1982.

18 Mossie K, Jallal B, Alves F, Sures I, Plowman GD, Ullrich A. Colon carcinoma kinase-4 defines a new subclass of the receptor tyrosine kinase family. Oncogene 11:2179–2184;1995.
19 Robinson D, He F, Pretlow T, Kung H-J. A tyrosine kinase profile of prostate carcinoma. Proc Natl Acad Sci USA 93:5958–5962;1996.
20 Schultz SJ, Nigg EA. Identification of 21 novel human protein kinases, including 3 members of a family related to the cell cycle regulator nima of *Aspergillus nidulans*. Cell Growth Different 4:821–830;1993.
21 Shimizu Y, Weidmann E, Iwatsuki S, Herberman RB, Whiteside TL. Characterization of human autotumor-reactive T-cell clones obtained from tumor-infiltrating lymphocytes in liver metastasis of gastric carcinoma. Cancer Res 51:6153–6162;1991.
22 Shu HK, Pelley RJ, Kung HJ. Tissue-specific transformation by epidermal growth factor receptor: A single point mutation within the ATP-binding pocket of the erbB product increases its intrinsic kinase activity and activates its sarcomagenic potential. Proc Natl Acad Sci USA 87:9103–9107;1990.
23 Takahashi T, Shirasawa T, Miyake K, Yahagi Y, Maruyama N, Kasahara N, Kawamura T, Matsumura O, Mitarai T, Sakai O. Protein tyrosine kinases expressed in glomeruli and cultured glomerular cells: FLT-1 and VEGF expression in renal mesangial cells. Biochem Biophys Res Commun 209:218–226;1995.
24 Uchino S, Tsuda H, Maruyama K, Kinoshita T, Sasako M, Saito T, Kobayashi M, Hirohashi S. Overexpression of c-erbB-2 protein in gastric cancer. Its correlation with long-term survival of patients. Cancer 72:3179–3184;1993.
25 Wilks AF. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci USA 86:1603–1607;1989.
26 Wilks AF, Kurban RR, Hovens CM, Ralph SJ. The application of the polymerase chain reaction to cloning members of the protein tyrosine kinase family. Gene 85:67–74;1989.
27 Wu C-W, Hsieh M-C, Lo S-S, Tsay S-H, Lui W-Y, P'eng F-K. Relation of number of positive lymph nodes to the prognosis of patients with primary gastric adenocarcinoma. Gut 38: 525–527;1996.
28 Wu C-W, Wang S-R, Chien S-L, Yeh T-H, Lian S-L, Shimizu N, Lui W-Y, P'eng F-K, Chi C-W. Regulation of arginase production by glucocorticoid in three human gastric cancer cell lines. Life Sci 51:1355–1361;1992.
29 Yokota J, Yamamoto T, Miyajima N, Toyoshima K, Nomura N, Sakamoto H, Yoshida T, Terada M, Sugimura T. Genetic alterations of the c-erbB-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-erb A homologue. Oncogene 2:283–287;1988.