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NTAKα and β Isoforms Stimulate Breast Tumor Cell Growth by Means of Different Receptor Combinations[1]

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Neural- and thymus-derived activator for ErbB kinases (NTAK) is a recently described member of the neuregulin family that binds directly to ErbB3 and ErbB4 and transactivates ErbB2. Rat NTAK has at least five alternative-spliced isoforms: α1, α2a, α2b, β, and γ. In order to understand their biological properties, this study focused on the NTAKα2a and β isoforms, which have different EGF-like domains. The effect of these isoforms on cell growth and tyrosine phosphorylation in human breast cancer cells, MDA-MB-453 and T47D, was examined using the recombinant proteins. In terms of cell growth, NTAKα2a and NTAKβ preferentially stimulate T47D cells and MDA-MB-453 cells, respectively, in a dose-dependent manner. Although both NTAKs induce the highest level of tyrosine phosphorylation of ErbB2, NTAKα2a and NTAKβ preferentially induce ErbB3 and ErbB4 phosphorylation, respectively. Thus, NTAKα2a and NTAKβ stimulate cell growth in different ways, by means of different combinations of receptors.

Key words: EGF, ErbB, neuregulin-2, NTAK, tyrosine phosphorylation.

The ErbB family of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR or ErbB1), ErbB2 (neu), ErbB3, and ErbB4 (*1–4*), are important mediators of cell growth, differentiation, and survival (*5*, *6*). In an earlier report, we described the purification and cloning of a novel member of the epidermal growth factor (EGF) family, which we named the neural- and thymus-derived activator for ErbB kinases (NTAK) (*7*). NTAK is an alternative splicing isoform, which is derived from the same gene as neuregulin-2 (NRG2) and divergent of a neuregulin-1 (Don-1) (*8–10*). NTAK is structurally homologous to neuregulin-1 (NRG1) in terms of its immunoglobulin (Ig)-like, EGF-like, and hydrophobic domains. The amino acid sequences of NTAKα2a and NTAKβ differ only in the third disulfide loop of the EGF-like domain (Fig. 1), suggesting a diversity of target receptor specificity and biological activity. NTAKα and NRG2α share the same EGF-like domain, but the amino acid sequence of the subsequent juxtamembrane domain is different. NTAKβ and NRG2β have the same EGF-like and α/β domains, and NRG2β contains an additional sequence in the variable region, compared with NTAKβ. NTAK is produced in the form of a transmembrane-anchored glycoprotein precursor and is secreted as a mature 46-kDa form. NTAK binds directly to ErbB3 and ErbB4 and transactivates ErbB1 and ErbB2 *via* heterodimerization with ErbB3 or ErbB4 (*7*), in the same manner as NRG1. It has been reported that the ErbB2/ErbB3-expressing cells are stimulated by both NRG2α and NRG2β, whereas the ErbB2/ErbB4-expressing cells are stimulated by NRG2α to a greater extent than NRG2β, as evidenced by data collected on a series of 32D myeloid cells expressing all combinations of ErbBs in a reconstituting system (*11*). NRG2β binds ErbB4 in preference to ErbB3 and has higher affinities to ErbB2/ErbB3 and ErbB2/ErbB4 than does NRG2α (*12*). In the case of MDA-MB-453 cells, NRG2β as well as NRG1β stimulates tyrosine phosphorylation of ErbB2 and ErbB3, whereas in MDA-MB-468 cells NRG2β inhibits cell growth, *via* activation of EGF receptor (*13*). Another newly isolated family of NRG1-like ligands, termed neuregulin-3 (NRG3) and neuregulin-4 (NRG4), has recently been described (*14*, *15*). Both NRG3 and NRG4 bind ErbB4, but not ErbB1, ErbB2, or ErbB3.

ErbB2 remains with no direct ligand, but plays an important role in the heterodimerization of the other ErbB family and the tyrosine phosphorylation. While ErbB2 decelerates the rate of ligand dissociation, ErbB2-containing heterodimers prolong intracellular signals *via* interaction with all the ErbB ligands (*16*). This may be related to the observation that the overexpression of ErbB2 indicates a poor prognosis in breast and ovarian cancers (*17*).

EGF (*18*), transforming growth factor-α (*19*), amphiregulin (*20*), heparin-binding EGF-like growth factor (HB-EGF) (*21*), betacellulin (*22*), and epiregulin (*23*) are known to be ligands of ErbB1. In addition, NRG1 and NTAK are capa-

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Abbreviations: CHO, Chinese hamster ovary; EGF, epidermal growth factor; FCS, fetal calf serum; HRP, horseradish peroxidase; Ig, immunoglobulin; NRG, neuregulin; NTAK, Neural- and thymus-derived activator for ErbB kinases; PS, penicillin/streptomycin sulfate; RT-PCR, reverse transcriptase–polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

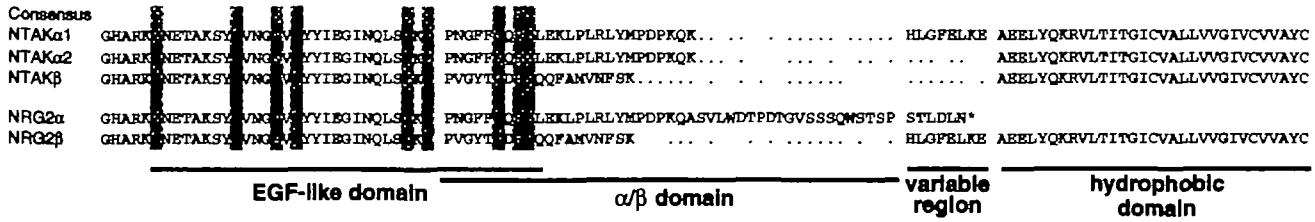

Fig. 1. **Isoform analyses of rat NTAK and NRG2.** The α and β isoforms of rat NTAK and NRG2 (*8*, *13*) are aligned. Asterisk, COOH-terminus Gaps are inserted in the sequence in order to provide the best alignment

ble of binding directly to ErbB3 and ErbB4. The mechanism by which the combination of ligand-receptor differentiates remains unknown. NRG1 exists in many isoforms, and the most variable region of these is the EGF-like domain with the transmembrane sequence. NRG1α isoforms are low-affinity ligands and are largely expressed in mesenchymal cells, while most of the NRG1β isoforms are located in neural cells. The differences in the role and function of the various NTAK isoforms are unknown. We report here that the α and β isoforms of NTAK bind to different receptors and stimulate cell growth in different manners in the human breast cancer cell lines MDA-MB-453 and T47D.

EXPERIMENTAL PROCEDURES

Materials—MDA-MB-453 and T47D cells were purchased from the American Type Culture Collection. MDA-MB-453 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM), 10% fetal calf serum (FCS), 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate (D-MEM/10% FCS/PS). T47D cells were maintained in RPMI 1640, 10% FCS and PS (RPMI/10% FCS/PS). Polyclonal antibodies to EGF receptor, ErbB2, ErbB3, and ErbB4 were purchased from Transduction Laboratories (Lexington, KY). Anti-NTAK antibody, #N1-1, was raised in rabbits against a synthetic peptide that corresponds to the 308–327 amino acid sequence (*7*).

Sample Preparation from NS-67 Cells—The NS-67 cell line was established from Chinese hamster ovary (CHO)-K1 cells by transfection with full-length NTAK rat cDNA subcloned into the pRc/CMV plasmid (Invitrogen, Groningen, Netherlands) (*7*). The conditioned medium of NS-67 cells cultured in Ham F-12 medium/2% FCS/PS was collected and supplemented with 0.25 M NaCl.

Sample Preparation from Escherichia coli—The 1.2-kbp *Bam*HI–*Bgl*II fragments of rat NTAKα2a and NTAKβ cDNAs, both of which correspond to extracellular regions, were inserted into PinPoint Xa-1 vectors (Promega, Madison, WI). The resulting plasmids were then used to transform *E. coli* DH5α, and the recombinant proteins were expressed by the addition of isopropyl-β-D-thiogalactoside (IPTG). The cells from 5-liter cultures were harvested 5 h after induction by centrifugation. The pellets were resuspended in 0.5 M NaCl/20 mM Tris-HCl, pH 7.2, containing 0.5 mM EDTA, 0.1 mM aprotinin, 20 μg/ml 3,4-dichloroisocoumarin, and 100 mM (*p*-amidinophenyl)methanesulfonyl fluoride HCl, and sonicated. After centrifuging at 15,000 rpm for 20 min, the supernatants were collected.

Purification of rNTAK—Recombinant NTAK was purified from the conditioned medium of NS-67 cells and *E. coli* extracts by heparin affinity, Cu-chelate, and C_4 reversed phase column chromatographies. The sample prepared as described above was applied to an AF-Heparin Toyopearl 650 M column (50 × 100 mm; Tosoh, Tokyo) equilibrated with 0.5 M NaCl/20 mM Tris-HCl, pH 7.2. After extensive washing with the equilibration buffer, the bound proteins were eluted batchwise with 1 M NaCl/20 mM Tris-HCl, pH 7.2 at a flow rate of 300 ml/h. The eluant was applied to a copper-chelating Sepharose column (50 × 150 mm; Amersham Pharmacia Biotech, Sweden) which had been saturated with cupric sulfate and equilibrated with 0.5 M NaCl/20 mM Tris-HCl, pH 8.0. After extensive washing with the equilibration buffer, the bound proteins were eluted batchwise with 0.15 M NaCl/70 mM imidazole/20 mM Tris-HCl, pH 8.0 at a flow rate of 300 ml/h. The eluant was loaded onto a C_4 reversed phase column (4.6 × 250 mm; nacalai tesque, Kyoto) equilibrated with 5% acetonitrile in 0.05% trifluoroacetic acid (TFA), using a Shimadzu LC-10A HPLC system (Shimadzu, Kyoto). The column was washed extensively with the equilibration solution, and the bound proteins were eluted with a 60-ml linear gradient of 20–60% acetonitrile in 0.05% TFA at a flow rate of 1 ml/min. The eluate was monitored by UV absorption at 280 and 214 nm. The active fractions were pooled, diluted with 1 volume of double-distilled, filtered water containing 0.05% TFA, and applied onto the second C_4 column. The bound proteins were eluted with a 90-ml linear gradient of 20–35% acetonitrile in 0.05% TFA. For all these procedures, the reaction tubes were siliconized with Sigmacote (Sigma-Aldrich Japan, Tokyo) to avoid activity loss as a result of non-specific absorption.

Detection of ErbBs mRNA by Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Using the TaqMan Method—MDA-MB-453 and T47D cells were homogenized in a TRIzol Reagent (Life Technologies, Frederick, MD), and their total cellular RNAs were extracted according to the manufacturer's instructions. Subsequently, mRNAs were isolated from the total RNA using an Oligotex-dT30 〈Super〉 mRNA purification kit (Takara Shuzo, Kyoto). The mRNA expression level of the ErbB family in both cells was quantified by PCR with the TaqMan method (*24*) and the GeneAmp 5700 Sequence Detection System (PE Biosystem, Foster City, CA). RT-PCR reaction was started with an initial reverse transcription step at 48°C for 30 min. After polymerase activation at 95°C for 10 min, PCR was performed for 40 cycles of 95°C for 15 s and 60°C for 1 min. The following oligonucleotide primers were prepared: ErbB1 (sense 5′-GCCACCCATATGTACCATCGAT-3′, anti-sense 5′-TTCGATGATCAACTCACGGAAC-3′), ErbB2 (sense 5′-TGCTGGAGGACGATGACATG-3′, anti-sense 5′-CTGGACAGAAGAAGCCCTGC-3′), ErbB3 (sense 5′-GCGCCGGATTCAGAATAAAA-3′, anti-sense 5′-GCTTCCTT-

AGCTCTGTCTCTTTGAAG-3′), and ErbB4 (sense 5′-CTTCAAGCATTGGATAATCCCG-3′, anti-sense 5′-TGGCTCATTCACATACTCATCCTC-3′). Every PCR reaction also contained 100 nM of each probe (ErbB1: 5′-Fam-TGCTGGATGATAGACGCAGATAGTCGCC-Tamra-3′, ErbB2: 5′-Fam-CTGGTGGATGCTGAGGAGTATCTGGTACCC-Tamra-3′, ErbB3: 5′-Fam-CCCAGTGAGAAGGCTAACAAAGTCTTGGC-Tamra-3′, and ErbB4: 5′-Fam-ATCACAATGCATCCAATGGTCCACCC-Tamra-3′; purchased from PE Biosystems). Amplification of the PCR product of each ErbB mRNA was determined with a standard curve using ErbB2.

Cell Growth Assay—For the assay of cell number, cells were resuspended in the maintenance medium, then seeded onto 12-well microplates (2×10^3 cells/well/1 ml). The plates were incubated for 12 h at 37°C, then refed with a medium containing 2% FCS. After 12 h, the samples to be tested were added. After 4 (T47D cells) or 5 days (MDA-MB-453 cells) of incubation, the cells were harvested and counted using a Coulter Counter (Coulter Electronics, Luton, England).

Immunoprecipitation and Western Blotting Analyses—Cells were cultured to subconfluence in the maintenance medium, then transfered to medium with 0.5% FCS. After a 12-h incubation, cells were harvested and resuspended in ice-cold medium with 0.5% FCS. Twenty ng/ml of NTAK was added, and cells were incubated for 12 min at 37°C. Cells were lysed with lysis buffer [1% Triton X-100, 1 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 5 mM EDTA, 0.1 mM aprotinin, 20 μg/ml 3,4-dichloroisocoumarin, 1 mM (*p*-amidinophenyl) methanesulfonyl fluoride HCl]. After centrifugation at 15,000 rpm for 10 min, supernatants were incubated with specific antibodies for 2 h at 4°C, then with 20 μl of protein A trisacryl (50% suspension; Pierce) for 2 h at 4°C. The samples were analyzed by electrophoresis on 6% polyacrylamide gels. Proteins in the gels were transferred to nitrocellulose membrane (Scheicher & Schuell, Dassel, Germany) in 150 mM 3-[cyclohexylamino]-1-propane-sulfonic acid (CAPS) buffer, pH 10.5, containing 20% methanol. The membrane was blocked with 5% skimmed milk in PBS(–) overnight at 4°C and incubated with anti-phosphotyrosine antibodies PY20 (Transduction Laboratories) in 5% skimmed milk/PBS for 4 h at room temperature. The membrane was washed three times at 10-min intervals with 0.05% Tween 20 in PBS, then incubated with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. The membrane was washed again three times at 10-min intervals with 0.05% Tween 20 in PBS, then incubated with avidin-HRP (Vectastatin, Vector Laboratories) for 30 min at room temperature. The membrane was washed five times with 0.05% Tween 20 in PBS, and HRP was detected by use of an ECL kit (Amersham Pharmacia Biotech) and autoradiography.

RESULTS

Functional Expression of Rat NTAK Isoforms in E. coli—To characterize the biological properties of NTAKα2a and NTAKβ, we purified the recombinant forms of NTAKα2a and β biotinylated tag proteins (bp-tagged NTAK) produced in the bacterial expression system using PinPoint Xa-1 vector. The purified bp-tagged NTAK appeared as two bards of 40 and 45 kDa. Western blotting analysis using an NTAK antibody (#N1-1) clearly showed that both bands were NTAK (Fig. 2A). To estimate the specific activities of the purified recombinant bp-tagged NTAKs, their ability to induce phosphorylation of total ErbBs in MDA-MB-453 cells was examined. Both showed maximal ErbB phosphorylation at 10 ng/ml, which was within the physiological concentration range (Fig. 2B). Their activities were comparable to that of rat NTAKα2a purified from NS-67 cells, suggesting that the glycosylation of NTAK is not essential for the activation of ErbB tyrosine kinases.

Biological Effects on MDA-MB-453 and T47D Cells—Before studying the biological effects of bp-tagged NTAKs on the human breast tumor cell lines, MDA-MB-453 and T47D, we estimated the expression levels of the ErbB family therein by quantitative RT-PCR analyses. Figure 3 shows that ErbB2, ErbB3, and ErbB4, which are major target receptors of NTAKs, were expressed in the both cell lines. The expression level of ErbB4 mRNA was higher than that of ErbB3 in MDA-MB-453 cells, whereas that of ErbB3 was higher than that of ErbB4 in T47D cells. To investigate the biological effects of NTAKα2a and NTAKβ, we first examined their induction of cell growth. Both

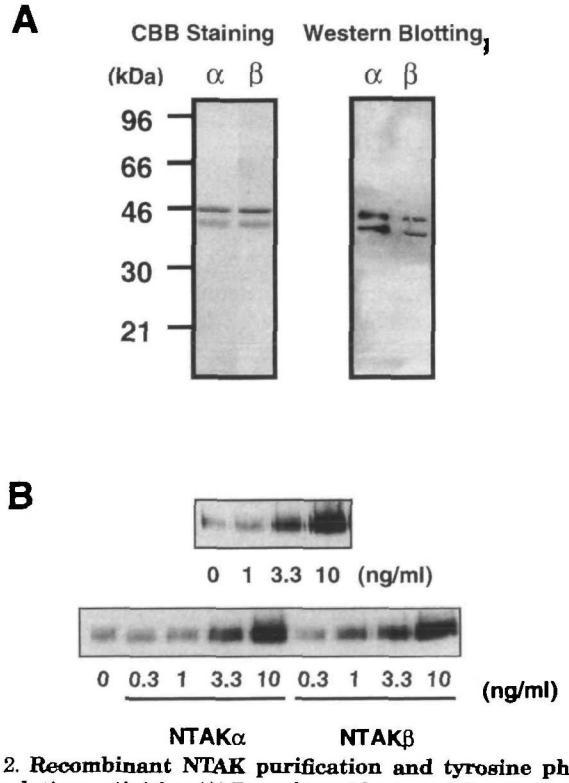

Fig. 2. **Recombinant NTAK purification and tyrosine phosphorylation activities.** (A) Recombinant bp-tagged NTAKα2a and NTAKβ purified from *E coli* were analyzed by 12% SDS-PAGE and stained with Coomassie Brilliant Blue (left panel), or subjected to Western blotting analysis (right panel) (B) MDA-MB-453 cells were incubated for 12 min at 37°C with increasing levels of recombinant NTAK purified from NS-67 cells (upper panel) or *E coli* (lower panel), and then lysed with 1% Triton-X 100 solution. The lysates were immunoprecipitated with anti-phosphotyrosine antibody, PY-20, then treated with protein A trisacryl The precipitated proteins were separated by 6% SDS-PAGE, transferred to nitrocellulose membranes, and detected by use of an ECL kit.

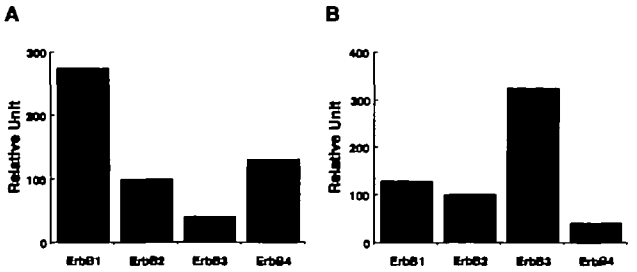

Fig. 3. **Quantitative analyses of mRNA transcripts of ErbBs by RT-PCR.** Cellular mRNA was extracted and isolated from MDA-MB-453 and T47D cells. ErbB mRNA expressions in MDA-MB-453 (A) and T47D cells (B) were measured by RT-PCR with the TaqMan method and the GeneAmp 5700 Sequence Detection System. The amount of PCR product of each ErbB mRNA was determined with a standard curve using ErbB2. Each amount was estimated from triplicate measurements.

NTAKα2a and NTAKβ stimulated growth in a dose-dependent manner. NTAKβ was more effective than NTAKα2a on MDA-MB-453 cells and showed maximal stimulation at 1 ng/ml (Fig. 4A). On the contrary, NTAK α2a stimulated T47D cell growth more than NTAKβ (Fig. 4C). We next examined the tyrosine phosphorylation of ErbBs by NTAKs. Both NTAKs induced the highest level of tyrosine phosphorylation in ErbB2 and moderate levels of phosphorylation of ErbB3 and ErbB4. In both cell lines, NTAKα2a preferentially activated ErbB3 to ErbB4, and NTAKβ showed the opposite preference (Fig. 4, B and D).

DISCUSSION

NTAK has recently been described as a member of the EGF family that binds directly to ErbB3 and ErbB4 (*7*). As shown schematically in Fig. 1, NTAK exists in two major isoforms, α and β, which have a sequence divergence in the third cysteine loop of the EGF-like domain, and NTAK represents alternative splicing isoforms derived from the same gene as NRG2 (*7–10*). NTAKα and NRG2α share the same EGF-like domain and have different amino acid sequences in the subsequent juxtamembrane domain. NTAKβ and NRG2β have the same EGF-like and α/β domains, and NRG2β has an additional sequence in the variable region, compared with NTAKβ. The differences between NRG2α and NRG2β have been reported (*11–13*). Here, we investigated the biological difference between NTAKα2a and NTAKβ in terms of cell growth and the tyrosine phosphorylation of ErbBs.

The recombinant bp-tagged NTAKs were produced in an *E. coli* expression system, and both purified proteins were found to have similar biological activity to the CHO-derived NTAK (*7*) in the physiological concentration range from 0.1 to 10 ng/ml. This suggests that the glycosylation of NTAK is not essential for the tyrosine phosphorylation of ErbBs. The NTAKα2a and β isoforms stimulated cell growth differently in two human breast cancer cell lines, MDA-MB-453 and T47D, both of which expressed ErbB1, ErbB2, ErbB3, and ErbB4 (Fig. 3). In the case of MDA-MB-453 cells, NTAKβ stimulated cell growth in a dose-dependent manner to a greater extent than NTAKα2a (Fig. 4A). On the contrary, NTAKα2a stimulated cell growth in a dose-dependent manner to a greater extent than NTAKβ in

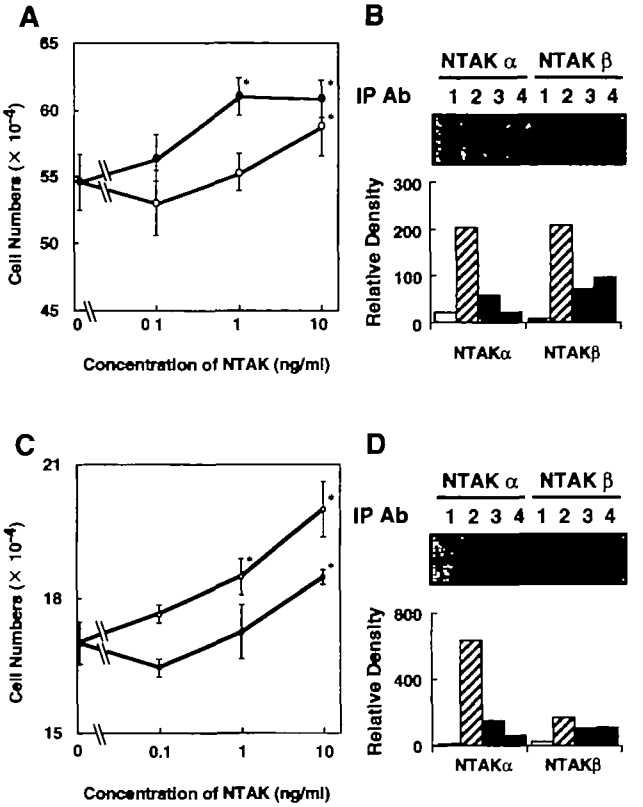

Fig. 4. **NTAK-stimulated cell growth and tyrosine phosphorylation of ErbB.** MDA-MB-453 cells (A) and T47D cells (C) were seeded on 12-well plates at the density of 2×10^3 cells/well, and after a 12-h incubation, the cells were refed with the medium containing 2% FCS in the presence of NTAKα2a (open circles) or NTAKβ (closed circles). Cells were counted after 4 (C) or 5 days (A) incubation. Each point is the mean of triplicate measurements. MDA-MB-453 cells (B) and T47D cells (D) were incubated for 12 min at 37°C with NTAKα2a (20 ng/ml) or NTAKβ (20 ng/ml), then lysed with 1% Triton-X 100 solution. The lysates were immunoprecipitated with anti-ErbB1, ErbB2, ErbB3, or ErbB4 antibody, then treated with protein A trisacryl. The precipitated proteins were separated by 6% SDS-PAGE, transferred to nitrocellulose membranes, and detected by use of an ECL kit (B, D: upper panels). The densities of the proteins were quantified using a densitometer (B, D: lower panels). Results are representative of three independent experiments. *$p < 0.05$

T47D cells (Fig. 4C). It has been shown that NRG2α stimulates the growth of 32D cells which express either ErbB3 or ErbB4 to a greater extent than NGR2β in a reconstituting system (*11*), and that NRG2β inhibited the cell growth of MDA-MB-468 cells, whereas NRG2α stimulated cell growth (*13*). NTAKα2a and NTAKβ had the opposite preference for the cell growth stimulation of MDA-MB-453 and T47D cells. Moreover, in terms of the tyrosine phosphorylation of ErbBs, NTAKα2a preferentially activated ErbB3 rather than ErbB4, while NTAKβ showed the opposite preference. At the same time, both NTAKs induced the highest level of tyrosine phosphorylation in ErbB2 among the ErbB family members examined. We reported earlier that NTAK binds directly to ErbB3 and ErbB4, but not to ErbB1 or ErbB2 (*7*). It has been suggested that both isoforms highly transactivated ErbB2 *via* ErbB3 or ErbB4. By quantitative RT-PCR analyses, the expression levels of ErbBs in the two

cell lines were estimated (Fig. 3). Both cell lines expressed ErbB2, ErbB3, and ErbB4. In MDA-MB-453 cells, ErbB4 expression was higher than those of ErbB2 and ErbB3, whereas in T47D cells, ErbB3 expression was higher than those of ErbB2 and ErbB4. Thus, the levels of ErbB phosphorylation and the mitogenic response stimulated by different isoforms of NTAK did correlate to the expression levels of either ErbB3 or ErbB4 in MDA-MB-453 and T47D cells. It has been reported that the N-terminal portion of the EGF-like domain of NRG1 allowed high-affinity and narrow-specificity binding to a primary receptor, whereas the variable C-terminal portion of the EGF-like domain, in which NRG1 isoforms had different sequences, binds to a secondary receptor with low affinity and broad specificity, making a heterodimer or homodimer (*25*). The sequence divergence in the third cysteine loop of the EGF-like domain could lead to a preference in the target ErbBs as experiments with NRG1 have shown (*6*). Both NRG2β and NTAKβ showed preferential binding to ErbB4, while ErbB3 did not. Unlike to NTAKα2a, NRG2α also showed preferential binding to ErbB4 rather than ErbB3. In addition, NRG2β had a higher affinity for the heterodimers of ErbB2/ErbB3 and ErbB2/ErbB4 than did NRG2α (*12*). The specificity of NTAK(NRG2)-ErbB interaction might be dependent on differences in the α/β domain sequence and the existence of the variable region in these factors (Fig. 1). The NTAKs used here contained the full-length extracellular region, which contained the Ig-like and EGF-like domains, whereas the NRG2s had only the EGF-like domain (*11–13*). The function of the Ig-like domain of NTAK remains unknown, but the Ig-like domain of NRG1 has a heparin-binding site and regulates the binding of NRG1 to ErbBs (*26*). The Ig-like domain of NTAKs would also interact with glycosaminoglycans on the cell surface and affect the interaction of NTAKs with ErbBs (*7*). The structure of both the Ig-like and EGF-like domains of the NTAKs would be essential for the interaction with ErbBs and the stimulation of cell growth. In all cases of NTAK-stimulated cell growth, ErbB2 was the most extensively phosphorylated of the ErbBs (Fig. 4, B and D). It is possible that all combinations of heterodimer and homodimer among ErbB2, ErbB3, and ErbB4 were formed, since the phosphorylation of all the ErbBs except ErbB1 was detected. NTAKα2a and NTAKβ preferentially phosphorylate ErbB3 and ErbB4, respectively. In MDA-MB-453 cells expressing ErbB4 more highly than ErbB3, NTAKβ stimulated cell growth to a greater extent than did NTAKα2a. On the contrary, NTAKα2a stimulated cell growth to a greater extent than NTAKβ in T47D cells expressing ErbB3 more highly than ErbB4. The level of cell growth stimulated by NTAK would be correlated to the total level of phosphorylation of ErbBs, with ErbB2 being phosphorylated the most extensively, suggesting that a combination of ErbB phosphorylation might be important in the regulation of cell growth and differentiation.

In summary, NTAKα2a and NTAKβ preferentially stimulated the growth of T47D cells and MDA-MB-453 cells, respectively, in a dose-dependent manner. NTAKα2a and NTAKβ showed different preferences in terms of the phosphorylation of ErbB3 and ErbB4, respectively. Further studies using all four isoforms will be needed to reveal the relation between NTAK isoform-ErbB combinations and their biological functions.

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