Nickel(II) induces alterations in EGF- and TGF-β1-mediated growth control during malignant transformation of human kidney epithelial cells

Steen Møllerup, Edgar Rivedal, Lovise Mølme and Aage Haugen

Department of Toxicology, National Institute of Occupational Health, PO Box 8149 Dep, N-0033 Oslo and Laboratory for Environmental and Occupational Cancer, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway

We have previously described immortalization of normal human kidney epithelial cells by nickel(II) and the subsequent tumorigenic conversion by v-Ha-ras transfection. We report here that nickel(II) induces alterations in growth regulatory control. Normal human kidney epithelial cells (NHKE) were growth inhibited by transforming growth factor β1 (TGF-β1). This effect was abrogated in both the immortalized (IHKE) and transformed (THKE) cells. NHKE expressed ~4700 high-affinity binding sites/cell for TGF-β1. IHKE and THKE showed reduced binding of 47% and 44% relative to NHKE respectively. On the other hand, expression of epidermal growth factor (EGF) receptors was elevated in IHKE (260%) and THKE (236%) relative to NHKE, which expressed 1.5 X 10^6 receptors/cell. Preincubation of IHKE and THKE with TGF-β1 resulted in reduced EGF binding, whereas this binding was unaltered in NHKE. Exposure of human kidney epithelial cells to EGF led to tyrosine phosphorylation of the EGF receptor and other cellular proteins in the mol. wt range from 42 to >300 kDa. The level of receptor phosphorylation induced by EGF reflected receptor expression. Tyrosine phosphorylated proteins appear to be identical in all three cell lines, and reach phosphorylation maxima independently of EGF receptor expression. These studies indicate that nickel carcinogenesis may involve changes in sets of genes important in normal growth regulation.

Introduction

Occupational exposure to nickel may result in cancer development (reviewed in 1). The mechanisms involved in the nickel-induced transformation process have not been defined. In mammalian cells, nickel compounds induce chromosomal aberrations, sister chromatid exchanges, DNA single-strand breaks and DNA-protein crosslinks (2). These effects are possibly mediated by reactive oxygen species (3,4). A model for nickel carcinogenesis has been proposed based on the binding of Ni(II) to nuclear proteins (5-7). Recently, nickel has been shown to disturb DNA nucleotide excision repair, possibly by inhibiting the incision step (reviewed in 8,9). We have demonstrated that treatment of human kidney epithelial cells during long-term culture with Ni(II) resulted in immortalized cell lines (IHKE*), and that nickel can induce mutation in the p53 tumor suppressor gene (10,11). We further showed that subsequent transfection of the IHKE cells with v-Ha-ras resulted in tumorigenic cells (12).

A variety of growth factors have been proposed to be involved in tumor development and in the increased proliferation of tumor cells. Carcinogenesis studies with mammalian epithelial cells have demonstrated altered response to growth factors and growth inhibitory factors, combined with altered expression of growth factor receptors (13-15). The epidermal growth factor (EGF) induces mitogenesis in virtually all epithelial cell types, mediated through binding to the 170 kDa ligand inducible EGF receptor (EGF-R) tyrosine kinase (reviewed in 16). Tyrosine kinase activity of the receptor is obligatory for signal transduction. The activated and dimerized receptor kinase phosphorylates tyrosine residues on the receptor itself and on substrate proteins involved in signal transduction (reviewed in 17-19). In vivo, enhanced expression of the EGF-R and the related erbB-2 oncogene is a common characteristic of human cancer cells (20-25). In addition, abnormal EGF-R expression has been described in renal cell carcinoma (26-28).

Transforming growth factor β (TGF-β) is a term covering a family of closely related multifunctional proteins, where TGF-β1 is the most thoroughly investigated form (29). TGF-β1 binds to specific membrane receptors, expressed by virtually all cells tested (30,31). Depending on the cell type, TGF-β affects differentiation, embryogenesis, and immunological and proliferative processes. TGF-β is a potent inhibitor of normal epithelial cell proliferation (29), arresting cells in the late G1 phase of the cell cycle (32). TGF-β modulates the expression of key regulatory genes involved in cell growth (33-36) and also influences post-translational modifications (32). During the carcinogenic process, tumor cells often lose the ability to respond to TGF-β in a growth-suppressive manner, and it has been proposed that loss of the anti-proliferative effect may be a necessary step for progression towards malignancy (37).

Recent studies have focused on the nickel-mediated DNA damage in various biological systems. There have been few studies that examine the role of growth factors during nickel-induced carcinogenesis. Therefore, this study was undertaken to determine whether immortalization of human kidney epithelial cells by nickel treatment and subsequent ras transformation induced alterations in growth control by EGF and TGF-β1.

Materials and methods

Cell lines and culture conditions

Normal human kidney epithelial (NHKE) cells were propagated by explant outgrowth culture (10). Early passages (passages 1-5) of primary cells were used in the experiments. Immortalized (IHKE) cells were derived by exposure of NHKE to NiSO₄ (10) and tumorigenic (THKE) cells were obtained by transfection of IHKE cells with v-Ha-ras (12). The kidney epithelial cell lines were cultured in DMEM/F12 (1:1) medium, supplemented with EGF (10 ng/ml), insulin (5 µg/ml), transferrin (5 µg/ml), 3,3',5-triiodothyronine (6.5 µg/ml), prostaglandin E₁ (25 ng/ml), hydrocortisone (36 ng/ml), Na-selenite (5 ng/ml) and 1% (IHKE and THKE) or 5% FCS (NHKE). NHKE cells and IHKE/THKE cells required 5% FCS and 1% FSC, respectively, for optimal growth. The cell lines were maintained at 37°C in humidified air containing
Receptor binding assays

EGF receptor binding was carried out in 24-well trays. Cells were seeded (1 x 10^5 per well) in DMEM/F12 + 5% (NHKE) or 1% FCS (IHKE and THKE) respectively, and cultured for 2 days. At this time the medium was replaced with fresh DMEM/F12 + FCS, with or without TGF-β1, as indicated, and incubation continued for 2 days. The plates were then incubated for 5 min on ice. The wells were washed with PBS containing 1 mg/ml BSA. Then, 200 μl of binding solution (MEM supplemented with 20 mM HEPES, pH 7.3, and 1 mg/ml BSA) were added, containing a fixed ratio of [125I]-labeled and unlabelled EGF. The plates were incubated for 6 h at 0°C. The fixed ratio of labeled and unlabelled EGF was used to minimize errors due to possible different binding affinities of the labeled/unlabeled EGF. Following incubation, 100 μl of the binding medium were collected from each well for determination of the free ligand concentration. The wells were washed with 4 x 1 ml ice-cold PBS/BSA; then 0.5 ml 0.5 M NaOH was added to each well and the plates were left for 10 min at room temperature prior to collection and counting of bound [125I]EGF. Non-specific binding (typically <5%) was determined using a 200-fold excess of unlabelled EGF, and verified to be proportional to the concentration of labeled ligand.

TGF-β1 receptor binding assay was carried out essentially as for EGF, with the following exceptions: 1 x 10^5 cells were seeded per well 3 days prior to the assay. Incubation with a fixed ratio of [125I]-labeled and unlabelled TGF-β1 was carried out for 2 h at room temperature (25°C). After washing of the wells with 4 x 1 ml PBS/BSA, 0.5 ml elution buffer containing 20 mM HEPES, pH 7.4, 1% Triton X-100 and 10% glycerol was added. The plates were placed on a shaker at room temperature for 10 min prior to collection and counting of the bound [125I]TGF-β1. Non-specific binding was determined using an 80- to 100-fold excess of cold TGF-β1, and verified to be proportional to the concentration of labeled ligand.

Binding data were analyzed using the INPLOT computer program (GraphPad, San Diego, CA). The binding constants were determined from non-linear regression of the binding data.

Preparation of cellular extracts for immunoblotting

Cells were seeded in 35 mm dishes at a density of 0.75-1.5 x 10^5 cells/dish in DMEM/F12 2 days prior to the experiment. Medium was replaced with fresh DMEM/F12 with the indicated concentrations of growth factors. [3H]Thymidine incorporation was measured by autoradiography at each concentration of TGF-β1, expressed as a percentage of untreated control. Each point corresponds to the mean of three estimations of acid-precipitable radioactivity at each concentration of TGF-β1, expressed as a percentage of untreated control. Error bars denote SD. *P < 0.01 and **P < 0.001 relative to untreated control in Student's t-test. Repeated experiments (at least three times) gave similar results.

Results

DNA synthesis—response to TGF-β1

The effect of TGF-β1 on cell proliferation, measured as tritiated thymidine incorporation into DNA, in normal, immortalized and tumorigenic human kidney epithelial cells, is shown in Figure 1. TGF-β1 inhibited thymidine incorporation in NHKE cells in the range of 0.05-1.0 ng/ml in a dose-dependent manner. The level of [3H]thymidine incorporation was significantly lower (P < 0.01 and 0.001) at concentrations from 0.1 to 1.0 ng/ml relative to untreated NHKE cells. On the other hand, the IHKE and THKE cells showed a marked loss of the inhibitory response to TGF-β1, and no significant differences from the untreated controls were observed at any concentrations in these cell lines. The experiments were carried out with competition of 10 mM P-Tyr, phosphoserine or phosphothreonine respectively.

Fig. 1. Effect of TGF-β1 on DNA synthesis. Human kidney epithelial cells (○, NHKE; ●, IHKE; ○, THKE) were incubated in the presence of TGF-β1 for 48 h prior to measurement of [3H]thymidine incorporation. Each point corresponds to the mean of three estimations of acid-precipitable radioactivity at each concentration of TGF-β1, expressed as a percentage of untreated control. Error bars denote SD. *P < 0.01 and **P < 0.001 relative to untreated control in Student's t-test. Repeated experiments (at least three times) gave similar results.

Fig. 2. Binding curves for TGF-β1 on NHKE (○), IHKE (●) or THKE (△) cells. Each point is the mean of two estimations of bound [125I]TGF-β1 at each concentration. Error bars denote SD. Values for Bmax and Kd were determined by non-linear regression analysis of the binding data. The experiments were repeated twice with similar results.
out under optimal growth conditions, i.e. in the presence of 10 ng/ml EGF, and 5% (NHKE cells) or 1% (IHKE and THKE cells) FCS. Omission of EGF from the culture medium during incubation with TGF-β1 did not cause any effect on the degree of inhibition of thymidine incorporation by TGF-β1 in the three cell lines (data not shown). Parallel experiments with measurement of cell proliferation by cell counting gave similar results (data not shown).

TGF-β1 binding

To determine if the loss of proliferative response by IHKE and THKE cells could be due to loss of TGF-β receptor, we carried out TGF-β1 binding experiments. The kidney epithelial cell lines were incubated in the presence of [125I]-labeled TGF-β1, and the bound radioactivity was measured as described in Materials and methods. Binding curves are shown in Figure 2. NHKE cells showed a maximal binding of 7.8 fmol/mg cellular protein (modifying to ~4700 TGF-β1 binding sites per cell), with a dissociation constant (K_D) of 52 pM. We found similar dissociation constants for IHKE and THKE cells, but maximal TGF-β1 binding was reduced to 3.7 and 3.4 fmol/mg protein respectively (2230 and 2050 binding sites/cell). Scatchard analysis of the data revealed that the kidney epithelial cell lines express a single high-affinity class of TGF-β1 binding sites. The TGF-β1 binding results are summarized in Table I.

EGF binding

NHKE cells are dependent on culture medium (DMEM/F12) containing EGF and FCS for in vitro growth. On the other hand, the IHKE and THKE cell lines proliferate in medium without FCS and EGF, and are only slightly stimulated by EGF (data not shown). Whether the altered growth requirement for EGF was reflected in EGF receptor expression was addressed in EGF-binding experiments. In a radioligand assay similar to that of TGF-β1, we measured binding of [125I]-labeled EGF to the kidney cell lines. The results from these experiments are shown in Figure 3(A,C,E). NHKE cells bound EGF with a maximum of 244.3 fmol/mg, corresponding to ~147 X 10^3 EGF receptors/cell. The IHKE and THKE cell lines showed enhanced binding of EGF, corresponding to 383 X 10^3 receptors/cell (260% relative to NHKE), and 348 X 10^3 receptors/cell (236% of NHKE). For all the cell lines, Scatchard analysis of the data indicated a single EGF receptor class with K_D of 1.36 nM (NHKE, Figure 3B), 0.22 nM (IHKE, Figure 3D) and 0.84 nM (THKE, Figure 3F).

Also shown in Figure 3 is the result of experiments where the human kidney epithelial cell lines were incubated in the presence of 1 ng/ml TGF-β1 for 48 h prior to determination of [125I]EGF binding. For the NHKE cells the binding data were similar to those obtained in the absence of TGF-β1, with virtually unaltered maximal binding. In contrast, TGF-β1 preincubation of the IHKE and THKE cell lines resulted in reduced maximal EGF binding. In IHKE cells the reduction in B_max was 15%, although this was not statistically significant (P = 0.051 in Student’s t-test). In THKE cells a reduction of 22% in B_max was found (P = 0.03). The reduction in maximal EGF binding in TGF-β1-exposed IHKE and THKE cells was generally observed in repeated experiments. A minor decrease in K_D for EGF binding in NHKE and THKE cells exposed to TGF-β1 relative to untreated cells could be seen in some experiments. In other experiments, however, the K_D for all three cell lines tended to be unaltered by TGF-β1 exposure. The EGF-binding data are summarized in Table II.

EGF receptor protein tyrosine kinase activity—response to EGF

Figure 4 shows the results of experiments carried out to evaluate the tyrosine kinase activity of the EGF-R in IHKE

| Table I. Binding of TGF-β1 to human kidney epithelial cell lines |
|-----------------|-----------------|-----------------|
| Cell line       | K_D (pM)        | B_max (fmol/mg) |
| NHKE            | 51.9±7.7        | 7.8±0.4         | 4700±240  |
| IHKE            | 64.2±15.3       | 3.7±0.3         | 2230±180  |
| THKE            | 75.7±34.1       | 3.4±0.6         | 2050±360  |

| Table II. EGF-R expression in human kidney epithelial cell lines in the absence or presence of TGF-β1 exposure |
|-----------------|-----------------|-----------------|
| Cell line       | TGF-β1          | B_max (fmol/mg) |
| NHKE            | 1.36±0.09       | 244.3±6.6       | 147         |
| IHKE            | 1.03±0.13       | 244.0±11.8      | 147         |
| THKE            | 0.22±0.04       | 634.8±26.5      | 383         |

*P < 0.05 in Student's t-test compared to TGF-β1-unexposed THKE cells.
cells. EGF was added to the medium of subconfluent cultures at increasing concentrations for a fixed time period (Figure 4A) or a fixed concentration for various lengths of time (Figure 4C). Tyrosine phosphorylated proteins were detected in Western blots with an anti-P-Tyr antibody as described in Materials and methods. Densitometric scanning of the P-Tyr band corresponding to the EGF-R (Figure 4A) is shown in Figure 4(B). Increasing concentrations of EGF led to increasing tyrosine phosphorylation on the EGF-R in the range of 0.1-100 ng/ml EGF. Proliferation of the human kidney epithelial cell lines reached maximum in the range of 3-10 ng/ml EGF, and decreased towards the level of untreated controls at higher concentrations of EGF (data not shown). Apparently, EGF-R tyrosine phosphorylation did not correlate with growth rate of the cells. Several other cellular proteins phosphorylated on tyrosine were detected. These proteins appeared to reach phosphorylation saturation at EGF concentrations submaximal for receptor phosphorylation, though at different EGF concentrations for the different proteins.

EGF rapidly induced tyrosine phosphorylation of the EGF-R, reaching maximum after 0.5 min (Figure 4C). Thereafter the P-Tyr level decreased to a very low level at 2 h, and after 6 h virtually no EGF-R P-Tyr could be detected. The kinetics of the receptor phosphorylation is shown in the densitometric scanning in Figure 4(D). Induction of tyrosine phosphorylation differed among the individual cellular substrate proteins: some reached maximal P-Tyr at 0.5 min and showed a relatively rapid dephosphorylation; others had later occurring maximum and concomitant later dephosphorylation. NHKE and THKE cells revealed similar tyrosine phosphorylation patterns to the IHKE cell line regarding EGF concentration and time of EGF incubation (data not shown).

A comparison of the EGF-induced protein tyrosine phosphorylation in different human kidney epithelial cell lines is shown in Figure 5. Tyrosine phosphorylation was induced on proteins with apparent mol. wts of 290-360, 230-240, 175 (EGF-R), 117, 100, 74, 57, 52 and 42 kDa. The spectrum of tyrosine phosphorylated proteins appeared to be quite similar in the NHKE, IHKE and THKE cells, with the possible exception of the 100 kDa protein not detected as tyrosine phosphorylated in NHKE cells. The P-Tyr level of the substrate proteins also appeared similar. However, densitometric scanning of Figure 5 demonstrated that the EGF-induced tyrosine phosphorylated band corresponding to the EGF-R was increased ~10- and 7-fold in the IHKE and THKE cells, respectively, compared to NHKE, reflecting the increased EGF-R expression in these cell lines.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** EGF-induced protein tyrosine phosphorylation. IHKE cells were exposed to increasing concentrations of EGF for 4 min or 200 ng/ml EGF at increasing times. EGF concentrations in (A) were: (1) without EGF; (2) 0.1; (3) 0.3; (4) 1; (5) 3; (6) 10; (7) 30; (8) 100; (9) 500 ng/ml EGF. Time-period of EGF incubation (C): (1) without EGF; (2) 0.5; (3) 1; (4) 2; (5) 5; (6) 15; (7) 60; (8) 120; (9) 360 min. Immunoblots of cellular extracts were probed with an anti-P-Tyr antibody. 'EGF-R' denotes position of the EGF receptor. Densitometric scanning of the band corresponding to the EGF-R are shown for the EGF concentration (B) and time experiments (D).

![Fig. 5](https://example.com/fig5.png)

**Fig. 5.** Comparison of EGF-induced protein tyrosine phosphorylation in NHKE (1 and 2), IHKE (3 and 4) and THKE cells (5 and 6). Cells were exposed to 200 ng/ml EGF for 5 min (2, 4 and 6). Immunoblot of cellular extracts was carried out as in Figure 3. 'EGF-R' denotes position of the EGF receptor.
Discussion

Nickel carcinogenesis is considered to involve binding of nickel ions to heterochromatin, causing covalent binding of amino acids to DNA through reactive oxygen intermediates (reviewed in 7,42). As a consequence, deletion or inactivation of senescence genes may take place (43). Nickel has also been shown to impair DNA nucleotide excision repair, thereby rendering the cell more susceptible to DNA-damaging agents (44). We previously reported that long-term exposure of human kidney epithelial cells to nickel resulted in chromosomal aberrations and p53 gene point mutation (T → C transition in codon 238) (10,11), indicative of the involvement of tumor suppressor gene(s) in nickel carcinogenesis. Generally, however, nickel is considered to have a rather low mutagenic potential, and a recent study of rat renal mesenchymal tumors induced by nickel revealed no (0/10 cases) p53 gene mutation (45). Yet, specific GGT → GTT transversion of the K-ras oncogene was demonstrated in nickel-induced rat renal sarcomas (46). In another study, nickel transformation of human osteoblasts resulted in loss of phosphorylation of the retinoblastoma protein, and it was suggested that the Rb gene was mutated as a result of nickel exposure (47). Taken together, results indicate that activation of oncogenes and/or inactivation of tumor suppressor genes may be involved in the neoplastic process induced by nickel.

Nickel exposure induced immortalization of NHKE cells, and v-Ha-ras transfection of the nickel-immortalized cells resulted in tumorigenesis in nude mice (10,12). The results presented in this study demonstrate that NHKE cells treated with Ni²⁺ ions in vitro have gained altered growth regulatory control. We report that immortalization by nickel was associated with altered responsiveness to TGF-β₁, resulting in abrogation of the inhibitory effect of TGF-β₁ on cell proliferation. Transfection of nickel-immortalized cells with the v-Ha-ras gene did not lead to further alterations in TGF-β₁ response, indicating that loss of responsiveness to TGF-β₁ may be an early event in in vitro carcinogenesis. Other studies have shown that TGF-β₁ might be involved at later stages of neoplastic development. The spontaneously immortalized human keratinocyte cell line (HaCaT) is growth inhibited by TGF-β₁, whereas the ras-transfected counterpart shows a decrease in response associated with increasing malignancy (14). A parallel of this study was reported by Reddel et al. (48), who showed that immortalized human bronchial epithelial cells were growth inhibited by TGF-β₁, which in these cells induced terminal differentiation. v-Ki-ras-transformed cells, on the other hand, were unaffected by TGF-β₁, both in respect of proliferation and differentiation. Similar observations were reported for rat tracheal epithelial cells (15). In this case, primary and preneoplastic cells were inhibited in their colony-forming ability by TGF-β, whereas tumor-derived cells were relatively resistant to the growth factor.

Evidence exists that mutant forms of the p53 protein may modulate the action of TGF-β₁. In a model of human thyroid follicular carcinogenesis, Wyllie et al. (49) observed a correlation between loss of responsiveness to the negative growth effect of TGF-β₁ and aberrant expression of p53 protein. Likewise, in a human bronchial epithelial cell line (BEAS-2B), transfection with a mutant human p53 cDNA (codon 143Val→Ala) resulted in reduced responsiveness to TGF-β₁, whereas wild-type-transfected cell lines showed increased responsiveness (50). These observations are in good agreement with the data presented here, showing that NHKE cells are growth inhibited, whereas immortalized and transformed cells, which possess a p53 point mutation and reveal increased expression of the p53 protein (11), are refractory to the negative growth effect of TGF-β₁.

We observed a correlation between loss of inhibitory response and reduction of TGF-β₁ binding sites in IHKE and THKE cells. In agreement with our data, induction of immortality in rat oral epithelial cell lines or ras-transformation of human keratinocytes resulted in reduced expression of TGF-β receptors (13,14). Reduced binding of TGF-β₁ is one of the mechanisms by which altered responsiveness to TGF-β₁ can be accomplished. However, post-receptor changes in TGF-β signaling may account for the TGF-β₁ inactivity. Studies have demonstrated that in vitro transformation of various cell types resulted in relatively small changes in TGF-β₁ binding (31). Transformation via retroviral infection and transfection with viral oncogenes caused a decrease in TGF-β₁ binding similar to our observations, whereas transformation by SV40 and chemically induced transformation resulted in increased binding. The concentration of TGF-β₁ required for half-maximal inhibition of DNA synthesis (~4 pM) in NHKE cells is considerably lower than the Kᵦ (52 pM) for TGF-β₁ binding, a result in agreement with Wakefield et al. (31), suggesting that only a minor fraction of the cellular binding sites have to be occupied for TGF-β₁ receptors to exert their growth inhibitory effects.

EGF receptors are found on normal and transformed cells of diverse tissue origin, including normal and neoplastic kidney cells. Generally, EGF induces mitosis in mammary cells of epithelial origin. Overexpression of the EGF-R is a frequent event in malignant cells of various origin, among these renal cell carcinomas (26–28). In the present study, we show that in vitro exposure of human kidney epithelial cells to the chemical carcinogen Ni²⁺ led to increased expression of EGF-R. We have previously reported chromosomal aberrations in the IHKE cells (10) involving also chromosome 7, where the EGF-R gene is located (51). In contrast, Game et al. (13) showed that immortalized and anchorage-independent tumorigenic rat oral cell lines expressed fewer EGF-Rs than their normal counterparts. In this study, expression of high-affinity binding sites for EGF correlated with tumorigenicity. We did not detect more than one class of binding sites in any of the kidney epithelial cell lines, even in binding experiments that employed very low concentrations of EGF (data not shown), indicating that high-affinity receptors are not a prerequisite for tumorigenic conversion.

TGF-β₁ treatment resulted in reduced binding of EGF to IHKE and THKE cells, whereas NHKE cells were unaffected. This is in contrast to the growth-regulatory effect exerted by TGF-β₁ on the human kidney epithelial cell lines. Several papers have described modulations of EGF-R expression by TGF-β, demonstrating increased or decreased numbers of high-affinity receptors, or increased mRNA expression (52–55). In agreement with our data for IHKE and THKE cells, mouse palatal mesenchyme cells responded to TGF-β by decreased EGF binding (56). Finally, as in the case of NHKE cells, no effect of TGF-β on EGF binding was observed in A431 or Mv1Lu epithelial cell lines (57,58). The mechanism(s) involved in TGF-β modulation of EGF-R expression/binding appear to be rather complex in view of the contradictory results obtained with different cell lines.

We found that addition of EGF to the kidney epithelial cell lines led to tyrosine phosphorylation of the EGF-R in immuno-
blotting experiments. In addition, tyrosine phosphorylation was induced in several other proteins. Some proteins are phosphorylated concomitantly with receptor phosphorylation, whereas others are phosphorylated at later stages. In addition, disappearance of P-Tyr on substrate proteins takes place at various times after growth factor application. Our results indicate that the level of EGF-R tyrosine phosphorylation induced by EGF reflected EGF binding, both being elevated in IHKE and THKE cells. It is interesting to note that maximal P-Tyr level of individual substrate proteins was reached at EGF concentrations submaximal for EGF-R phosphorylation (0.1–10 ng/ml), and that this correlates with the EGF concentrations supporting maximal cell proliferation in the kidney cell lines. This indicates that the level of EGF-R tyrosine phosphorylation must be balanced. In fact, in A431 human carcinoma cells highly overexpressing EGF-R, addition of EGF to cell cultures inhibits proliferation (59).

In summary, we report that nickel immortalization results in growth-regulatory alterations in human kidney epithelial cells. Tumorigenic cell lines showed similar growth regulation as immortalized cells, indicating that alterations in response to EGF and TGF-β were apparently associated with the immortalization step.

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References

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