EFFECT OF TCDD ON TELOMERASE ACTIVITY OF SPONTANEOUSLY IMMORTALIZED KERATINOCYTES

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Introduction

Exposure to TCDD produces a variety of toxic effects that are species and tissue specific. In humans, TCDD is known to target the skin, where the most prominent endpoint is chloracne. TCDD does not form DNA adducts and is non-genotoxic, but it is a potent tumor promoter in the skin of athymic mice[1] and in the liver of female rats.[2] Although TCDD is known to act through a stereospecific receptor that binds to response elements found in many genes[3], the mechanisms of its toxicity and tumor promotion are still largely unknown. TCDD is extremely persistent in the environment and, when taken up by organisms, it is eliminated very slowly (half life of years). Cells cultured from affected organs, including keratinocytes from human skin, provide good subjects for studying TCDD toxicity and its mechanism of action.

Normal human cells in culture have a finite replicative potential. Keratinocytes, for instance, replicate for only ~150 generations before they senesce. Although human cells can be immortalized by infection with certain viruses[4], spontaneous immortalization is very rare. Thus, cell senescence can be seen as a well-guarded tumor suppressor mechanism. Senescence appears to be triggered by a mechanism that senses when telomeres, a repetitive sequence constituting the terminal section of linear chromosomes, reach a critically short length[5]. Telomere shortening occurs with each cell division as a consequence of DNA replication. Immortal cells, on the other hand, express telomerase, an enzyme that elongates telomeres and thus provides a mechanism to override cellular senescence. The override phenomenon is illustrated by the remarkable lifespan extension observed in fibroblasts transfected with hTER, the telomerase catalytic subunit[6]. Thus if TCDD modulates telomerase activity, then it could affect tumor promotion.

We have previously examined the effect of epidermal growth factor (EGF) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on telomerase activity of normal human epidermal cells (hEp) and the spontaneously immortalized SIK line[7]. These cells exhibited similarly low telomerase activity when cultured in the absence of EGF, where they grew poorly. EGF addition improved growth substantially for both hEp and SIK. Furthermore, telomerase activity increased as a consequence of EGF treatment, but only in the minimally deviated SIK line[8]. In such experiments TCDD alone was ineffective, but it suppressed the stimulatory effect of EGF on telomerase. The SIK line likely became immortalized as a consequence of its elevated telomerase activity. We speculate that TCDD could function as a late stage promoter in these cells by selecting for more neoplastic variants in which the telomerase activity, as in malignant cells, is constitutively expressed and thus independent of TCDD exposure.

Present work examines the effect of TCDD and EGF on telomerase activity in three newly derived spontaneously immortalized keratinocyte lines: SIKer, SIKly, and HFS2 cells. SIKer and SIKly were derived from the same ostensibly healthy skin sample as the original SIK line, while HFS2 was derived from unrelated fetal tissue. All of these spontaneously immortalized cell lines resemble normal human keratinocytes in many respects including morphology, growth factor dependence and their essentially normal differentiation. Through ~400 generations, the three new lines all displayed typically low colony forming efficiencies (2-7% CFE). After this period of growth, the CFE increased to values characteristic for each cell line (~35% for SIKer,

~5% for SIKLY, and ~25% for HFS2) and were considered spontaneously immortalized. As in the case of the SIK line, late passages of SIKer and HFS2 cells had accumulated a small number of chromosomal aberrations, but SIKly cells retained a normal karyotype.

Materials and Methods

Cell culture. Cells were cultured by the standard 3T3 feeder layer system[9] using medium consisting of a 3:1 mixture of Dulbelcco-Vogt Eagle's and Ham's F-12 media supplemented with 0.4 μg/ml hydrocortisone, 5 μg/ml insulin, 5 μg/ml transferrin, 20 pM T_3, 0.18 mM adenine, 10 ng/ml cholera toxin and 5% fetal bovine serum. Treatment with EGF (10 ng/ml) or TCDD (10 nM) was initiated three days after plating and continued until harvest, usually after 10-14 days.

Telomerase assay. Telomerase activity was measured using the telomere repeat amplification protocol (TRAP)[10]. Briefly, cultures were trypsinized and 10^5 cells were lysed. An aliquot of cell lysate corresponding to 10^3 cells was incubated with a telomerase substrate for 30 min followed by PCR of telomerase-elongated products using ^{32}P labeled dCTP. PCR products were analyzed in 10% non-denaturing polyacrylamide gels, and telomerase activity was quantified by phosphorimaging of the radioactive gels. Telomerase activities were normalized to those of cultures not treated with EGF or TCDD. Results are from representative (hEp and SIK) or triplicate (SIKer, SIKly and HFS2) experiments with three repetitions each.

Results and Discussion

Present experiments addressed the question whether telomerase stimulation by EGF and suppression by TCDD are characteristic of spontaneously immortalized keratinocytes. As found for hEp and SIK cultures, the growth of SIKer, SIKly and HFS2 cells was dramatically improved by EGF addition to the culture medium. As previously reported[1], neither EGF nor TCDD affected telomerase activity in hEp (Figure 1A). In SIK cells, by contrast, EGF stimulated telomerase 3-4 fold, but simultaneous addition of TCDD (ineffective alone) largely prevented this stimulation (Figure 1C). In the related SIKer line, EGF stimulation of telomerase was also dramatic, 5-fold the level in untreated cells, reproducibly higher than in SIK cultures, but TCDD essentially prevented the stimulation in these cells also (Figure 1D). On the other hand, EGF was ineffective in stimulating telomerase activity in HFS2 cultures, but TCDD treatment alone induced nearly a three-fold increase (Figure 1B). TCDD also induced a marginal, but reproducible, increase in telomerase activity in SIKly cultures, while EGF was ineffective (Figure 1E). Strikingly, independently of the cells used, concurrent treatment with EGF and TCDD antagonized the increase on telomerase seen with either agent alone (Figures 1B-E). We conclude that telomerase can be deregulated in different ways in spontaneously immortalized keratinocytes, stimulated by EGF in some lines and by TCDD in others, and that the signal transduction pathways of EGF and TCDD are interconnected in a mutually antagonistic way.

It is likely that the spontaneous immortalization of two keratinocyte lines studied herein (SIK and SIKer) resulted from telomerase deregulation. By stimulating their telomerase, EGF treatment probably extended their lifespan until variants with chromosomal aberrations (and as a consequence higher colony forming abilities) took over the populations. Loss of this EGF sensitivity by the SIKly subline (derived from the SIKer line shortly before the latter evolved chromosomal aberrations) raises the possibility this subline may eventually senesce unless it is rescued by a variant with elevated colony forming ability (and telomerase activity). We speculate that telomerase deregulation could be the result of congenital abnormalities, acquired mutations or other unknown events. Individuals with cells displaying such deregulation may constitute a rare sensitive population subject to elevated tumorigenesis. By contrast, the HSF2 line has become immortalized without EGF-stimulated telomerase. Nevertheless, even in this line, telomerase activity can be modulated by factors present in the cellular microenvironment. Agents that are

effective in various keratinocyte lines include TCDD, EGF, UV light (not shown), and perhaps other physical or chemical environmental factors. TCDD is of particular note because it is eliminated from the body very slowly and thus is likely to provide prolonged effects. We speculate that such modulation could act as a tumor promoting stimulus, either by increasing the lifespan of neoplastic cells until they sustain further genetic damage or by selecting for more neoplastic variants within a heterogeneous premalignant population. Whether telomerase modulation is restricted to human epidermal cells or spontaneously immortal lines remains to be determined.

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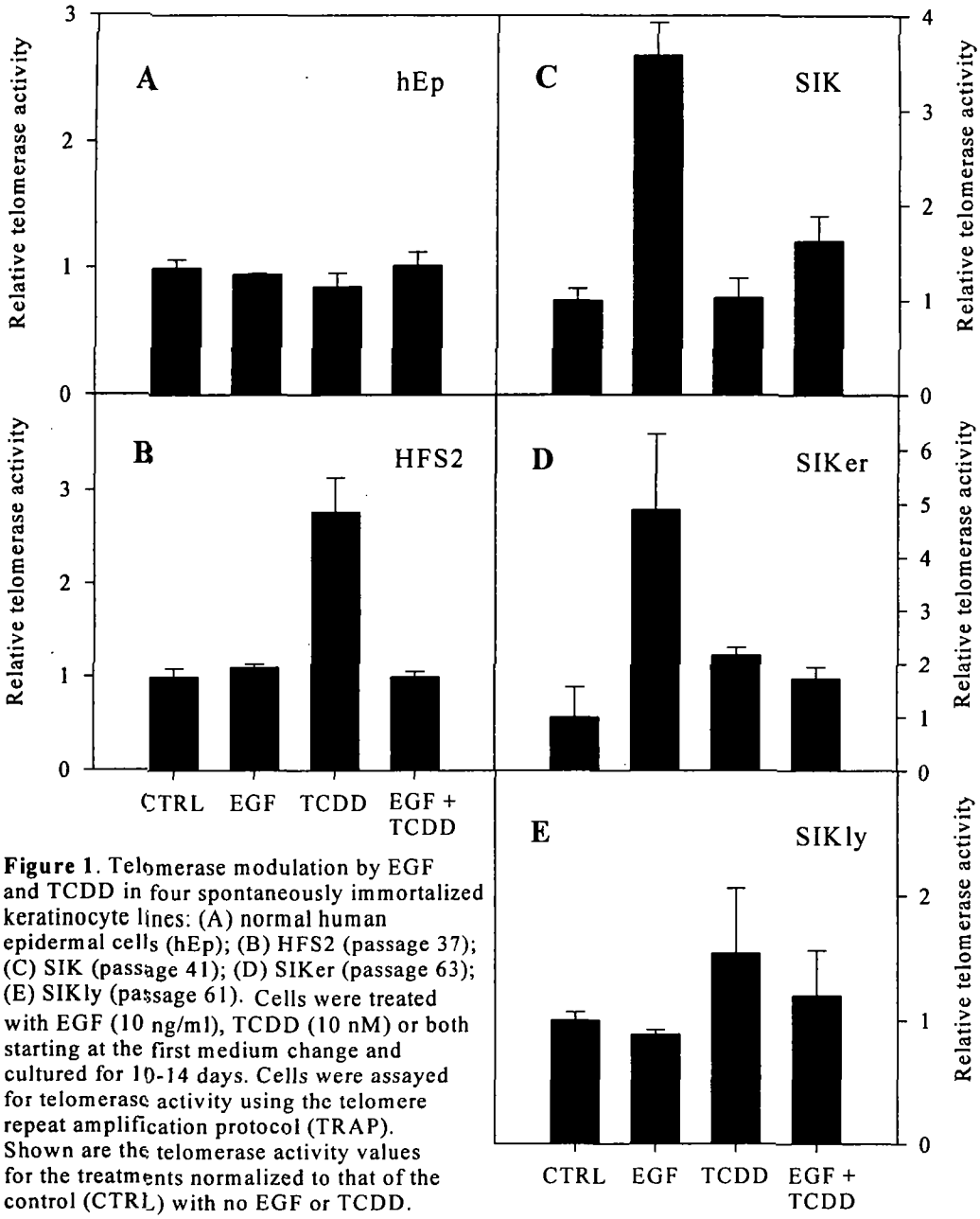

Figure 1. Telomerase modulation by EGF and TCDD in four spontaneously immortalized keratinocyte lines: (A) normal human epidermal cells (hEp); (B) HFS2 (passage 37); (C) SIK (passage 41); (D) SIKer (passage 63); (E) SIKly (passage 61). Cells were treated with EGF (10 ng/ml), TCDD (10 nM) or both starting at the first medium change and cultured for 10-14 days. Cells were assayed for telomerase activity using the telomere repeat amplification protocol (TRAP). Shown are the telomerase activity values for the treatments normalized to that of the control (CTRL) with no EGF or TCDD.