Evidence for the Epidermal Growth Factor Receptor As a Target for Lung Cancer Prevention

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Abstract

Purpose: There is a need to identify lung cancer prevention mechanisms. All-trans-retinoic acid (RA) was reported previously to inhibit N-nitrosamine-4-(methyl-nitrosamino)-1-(3 pyridyl)-1-butanone (NNK) carcinogenic transformation of BEAS-2B human bronchial epithelial cells (J. Langenfeld et al., Oncogene, 13: 1983–1990, 1996). This study was undertaken to identify pathways targeted during this chemoprevention.

Experimental Design: Because epidermal growth factor receptor (EGFR) overexpression is frequent in non-small cell lung cancers (NSCLC) and bronchial preneoplasia, BEAS-2B cells, carcinogen-transformed BEAS-2B\textsubscript{NNK}, and retinoid chemoprevented BEAS-2B\textsubscript{NNK RA} cells were each examined for EGFR expression. Whether RA treatment regulated directly EGFR expression or reporter plasmid activity was studied. RA effects on epidermal growth factor (EGF) induction of EGFR-phosphotyrosine levels, cyclin D1 expression and mitogenesis were examined in BEAS-2B cells.

Results: Findings reveal that NNK-mediated transformation of BEAS-2B cells increased EGFR expression. RA treatment repressed EGFR expression and reporter plasmid activity in these cells. This treatment reduced EGF-dependent mitogenesis as well as EGFR-associated phosphotyrosine levels and cyclin D1 expression. These findings extend prior work by highlighting EGFR as a chemoprevention target in the lung. Notably, RA treatment prevented transformation as well as outgrowth of EGFR overexpressing bronchial epithelial cells, despite NNK exposure. After acute NNK exposure, p53-induced species that appear after DNA damage or oxidative stress were evident before an observed increase in EGFR expression.

Conclusions: These findings indicate how effective chemoprevention prevents carcinogenic transformation of bronchial epithelial cells when repair of genomic damage does not select against EGFR overexpressing cells. This implicates EGFR as a chemoprevention target in the carcinogen-exposed bronchial epithelium.

Introduction

The EGFR and its ligands are important in normal and neoplastic epithelial cell growth. Diverse biological roles in malignancy are attributed to this autocrine growth factor loop, including regulation of mitogenesis, cell survival or apoptosis, angiogenesis, and cell motility or metastasis, making the EGFR an attractive therapeutic target, as reviewed previously (1). The EGFR has been implicated as a progression or prognostic factor in NSCLC, in which its overexpression is often detected (2–7). Aberrant EGFR expression is frequent in bronchial preneoplasia, suggesting that squamous cell cancer development and EGFR expression are related (8). This raised the prospect that exposure of human bronchial epithelial cells to tobacco-specific carcinogens would induce EGFR expression or activity early during squamous cell carcinogenesis. This is consistent with the view that EGFR is an intermediate marker or potential target for lung cancer prevention. Direct evidence for this had not existed previously for human bronchial epithelial cells.

This study was undertaken to determine whether exposure of human bronchial epithelial cells to a tobacco-specific carcinogen would induce EGFR expression or associated tyrosine kinase activity. Whether a chemopreventive agent blocked this induction was studied. An in vitro squamous cell carcinogenesis model was used where exposure to the carcinogen NNK was shown to transform BEAS-2B immortalized human bronchial epithelial cells (9). Notably, treatment with the chemopreventive agent all-trans-retinoic acid (RA) blocked this transformation process at least partly by triggering G\textsubscript{1} arrest via ubiquitin-dependent proteolysis of cyclin D1 (9–11). This is consistent with the view that cyclin D1 is a marker of RA chemopreventive response in human bronchial epithelial cells (11). Unique cell
lines were derived from BEAS-2B immortalized human bronchial epithelial cells (9). These included BEAS-2B_{NNK}, representing carcinogen-transformed BEAS-2B cells and BEAS-2B_{NNK RA}, isolated from BEAS-2B cells that were exposed to NNK and RA, where transformation was prevented (9).

These human bronchial epithelial cell models were used in the analysis of EGFR expression patterns after carcinogenic transformation. Whether RA treatment affected EGFR expression or its associated activities was studied. In these analyses, treatment with the EGF was used to confirm that in human bronchial epithelial cells RA prevented an EGF-dependent increase of EGFR-associated phosphotyrosine levels, induction of cyclin D1, and promotion of mitogenesis. To determine whether the observed increase in EGFR expression was an acute response to NNK treatment, the relationship was studied between EGFR expression and the induction of p21-associated species induced after DNA damage or oxidative stress. Findings that will be presented confirmed that EGFR-overexpressing cells were derived after carcinogenic exposure of human bronchial epithelial cells. Yet, RA treatment selected against outgrowth of these cells. These and other findings that will be presented provide evidence for the EGFR as a target for lung cancer prevention.

Materials and Methods

Human Bronchial Epithelial Cells. The BEAS-2B immortalized human bronchial epithelial cell line was derived from normal bronchial cells by immortalization with an adeno-virus 12-SV-40 hybrid virus, and this cell line was cultured in serum-free LHC-9 medium (Biofluids, Rockville, MD), as described previously (12). The BEAS-2B_{NNK} cell line was derived after exposure to the carcinogen NNK (9). The BEAS-2B_{NNK RA} cell line was derived after NNK exposure and continuous treatment with RA, as reported previously (9). These cells were passaged every 5–7 days and were used for experiments after 2–4 passages. Growth factor deprivation was achieved by culture of BEAS-2B cells for 6 days in LHC-9 medium without added growth factors or hormones. Acute NNK exposure was for 24 h at a dosage of 3 μg/ml. NNK was dissolved in DMSO as a vehicle, and as a control, cells were treated with the EGF was used to confirm that in human bronchial epithelial cells RA prevented an EGF-dependent increase of EGFR-associated phosphotyrosine levels, induction of cyclin D1, and promotion of mitogenesis. To determine whether the observed increase in EGFR expression was an acute response to NNK treatment, the relationship was studied between EGFR expression and the induction of p21-associated species induced after DNA damage or oxidative stress. Findings that will be presented confirmed that EGFR-overexpressing cells were derived after carcinogenic exposure of human bronchial epithelial cells. Yet, RA treatment selected against outgrowth of these cells. These and other findings that will be presented provide evidence for the EGFR as a target for lung cancer prevention.

Western Analysis. BEAS-2B or its derived human bronchial epithelial cells were rinsed twice with ice-cold PBS and then lysed on ice with Staph A buffer [1% Triton X-100, 0.1% SDS, and 0.5% deoxycholate, 0.01 m sodium phosphate (pH 7.4), 0.15 m NaCl], in the presence of proteinase and phosphatase inhibitors (2.5 μg/ml aprotinin, 2.5 μg/ml leupeptin, 2.5 μg/ml antipain, 250 μg/ml phenylmethylsulphonyl fluoride, and 10 mM sodium P). Protein concentrations of the clarified lysates were determined in duplicate using the Bradford assay (Bio-Rad, Hercules, CA). The purified proteins were size-fractionated by SDS-PAGE, transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) and the obtained membranes were reincubated in 5% nonfat milk in a Tris-buffered saline solution containing 0.1% Tween (Sigma Chemical Co., St. Louis, MO). EGFR protein was detected using a purchased anti-EGFR polyclonal antibody (Ab-4 anti-EGFR; Oncogene Science, Boston, MA) or (1005; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) that was incubated overnight at 4°C. After washings and use of an appropriate secondary antibody, detection was performed using the enhanced chemiluminescence system (Amersham, Arlington Heights, IL) and previously described techniques (10). Actin was detected in the desired immunoblots using a purchased antibody (C-11;Santa Cruz Biotechnology, Inc.). Western analyses were performed in triplicate independent experiments to confirm results.

Measurement of EGFR-associated Phosphotyrosine Levels. Relative EGFR-specific phosphotyrosine levels were measured in BEAS-2B, BEAS-2B_{NNK} and BEAS-2B_{NNK RA} cells that were cultured in LHC-8 medium without supplementation with EGF (Collaborative Biomedical Products/BD Discovery Labware, Bedford, MA). In brief, these assays were performed with minor modifications of a previously described assay (15). EGFR-associated phosphotyrosine levels were measured in these cells in response to EGF (50 ng/ml) treatments. Phosphotyrosine levels were detected using the PY54-HRP polyclonal antibody (Oncogene Sciences, Boston, MA) followed by addition of an antirabbit HRP antibody (Santa Cruz Biotechnology, Inc.). Duplicate experiments were conducted using differing amounts of total protein to confirm results. The total protein loaded was adjusted to permit a linear signal.

EGFR Scatchard Analysis. BEAS-2B cells were plated at 10^5 cells/well, and the following day, cells were rinsed with LHC basal media (16), supplemented with 2 mg/ml BSA as individually assessed using semiquantitative RT-PCR assays. Expression of these species was assessed using the following oligonucleotide primers: EGFR forward primer: 5’-GCACGAGTAA-CAAGCTCACG-3’ and EGFR reverse primer: 5’-GGA-ATTCCGTCCTACGTGT-3’; cyclin D1 forward primer: 5’-TCTCTCAAAAAATGCAAG-3’ and cyclin D1 reverse primer: 5’-TGAGGCCTAGTACAGG-3’; PIG3 forward primer: 5’-TTTGGCTGCACTTTGAC-3’ and PIG3 reverse primer: 5’-ATGCCCTAAGTCAATAATG-3’; P21 forward primer: 5’-GAGCGATGAACTTCGAC-3’ and P21 reverse primer: 5’-GGCTTCTCTTGGAGAAGAT-3’; and β-actin forward primer: 5’-GCGGGAAATGCTGGTGACA-3’ and β-actin reverse primer: 5’-AAGGAAGGCTGGAGATGC-3’. Each of these species was size-fractionated on an 0.8% agarose gel using standard techniques that were described previously (14). Northern analysis for EGFR expression was performed using previously described techniques (3).
binding buffer before room temperature incubation with varying concentrations of $^{125}$I-labeled EGF (Amersham). After incubation, cells were thoroughly rinsed with binding buffer, then were solubilized with 1% SDS, 10 mM NaOH and counted in a gamma counter. Nonspecific binding, for each point, was calculated by determining radioactivity bound in presence of a 100-fold excess of unlabeled EGF. The number of receptors per cell was determined from Scatchard plots using modifications of established techniques (17).

**Transient Transfection Experiments.** Transient transfection experiments were conducted using BEAS-2B cells. This was accomplished through independent transient transfection experiments in these cells using an Effectene (Qiagen Inc., Valencia, CA)-based transfection method with a cyclin D1 reporter plasmid, D1pro-1749 (18), or an EGFR reporter plasmid, pERCAT2 (19). For these reporter assay experiments, transcriptional activities were normalized to results obtained with a cotransfected β-galactosidase reporter plasmid.

**Results**

EGFR expression was examined in BEAS-2B, BEAS-2BNNK, and BEAS-2BNNK RA bronchial epithelial cells, as outlined in Fig. 1A. This figure indicates how exposure of BEAS-2B cells to NNK transformed these cells. This transformation led to increased EGFR expression in BEAS-2BNNK cells relative to BEAS-2B or to BEAS-2BNNK RA cells. This occurred for mRNA (data not shown) and protein levels of EGFR expression, as displayed in Fig. 1B. This followed a 24-h exposure of BEAS-2B cells to NNK and the subsequent passage of these cells without additional NNK treatments, as reported previously (9). RA treatment prevented transformation of BEAS-2BNNK RA cells (9) and blocked induction of EGFR expression in these cells, as confirmed by Western analysis for EGFR, as shown in Fig. 1B. This immunoblot analysis demonstrated an increased EGFR expression detected in BEAS-2BNNK as compared with BEAS-2BNNK RA or BEAS-2B cells. Scatchard analysis revealed a marked difference in EGFR number measured in BEAS-2BNNK versus BEAS-2BNNK RA cells (respectively, 24,000 as compared with 600 EGFR per cell).

As expected, this increase in EGFR expression in BEAS-2BNNK relative to BEAS-2BNNK RA or to BEAS-2B (data not shown) cells was associated with increased EGFR-associated phosphotyrosine levels, as shown in Fig. 2. The basal EGFR protein levels in BEAS-2BNNK RA cells were much lower than in parental BEAS-2B or BEAS-2BNNK cells. Basal EGFR-associated phosphotyrosine levels detected in EGF-untreated BEAS-2BNNK cells may be attributable to endogenously expressed EGFR ligand(s) that could act in an autocrine manner in these cells. Basal EGFR-associated phosphotyrosine levels were reduced in BEAS-2BNNK RA cells, and these cells had a markedly blunted response to EGF stimulation as compared with BEAS-2BNNK cells. As depicted in Fig. 2B, this is likely caused by the reduced levels of EGFR detected in BEAS-2BNNK RA relative to BEAS-2BNNK cells. These findings were indicative of reduced EGFR-specific tyrosine kinase activity present in BEAS-2BNNK RA relative to BEAS-2BNNK cells. These findings provide evidence for increased EGFR expression and activity after NNK exposure of human bronchial epithelial cells. These effects were antagonized by RA treatment.

Experiments were next undertaken to determine the relationship between carcinogen exposure of human bronchial epithelial cells and the increase in EGFR expression. Whether the observed increase in EGFR expression in BEAS-2BNNK cells directly followed NNK exposure was examined. To study this, BEAS-2B cells were exposed acutely to NNK (3 μg/ml) for 24 h. This exposure to NNK did not induce EGFR expression, as shown in Fig. 3. In contrast, NNK treatment led to a marked increase in expression of several p53-induced species associated with DNA damage or oxidative stress such as p21 or PIG3 (13). This induction was evident after acute exposure to NNK (Fig. 3). From these findings, it is apparent that the observed increase in EGFR expression in BEAS-2B cells is not immediate but subsequent to a DNA damage response of carcinogen-exposed human bronchial epithelial cells. RA treatment suppressed out-
growth of EGFR overexpressing human bronchial cells after DNA damage caused by the carcinogen NNK.

These findings were extended in several ways. It was reported previously that cyclin D1 and EGFR are often aberrantly expressed in bronchial preneoplasia (8, 20), which suggested that aberrant expression profiles of EGFR and cyclin D1 were related. Cyclin D1 has also been proposed as an intermediate marker of retinoid response in bronchial epithelial cells (11). For this reason, it was hypothesized that RA might affect expression of cyclin D1 after EGF-activation of the EGFR. It was proposed that EGF treatment would augment cyclin D1 expression and that RA treatment would prevent this induction of cyclin D1. This hypothesis was confirmed by findings displayed in Fig. 4. RA treatment prevented induction of cyclin D1 by EGF, as shown in Fig. 4A. EGF treatment of BEAS-2B cells was also mitogenic to these cells, but RA treatment prevented this growth stimulation of these cells, as displayed in Fig. 4B. Whereas findings presented in Fig. 1 and 2 indicated that exposure of human bronchial epithelial cells to a tobacco-specific carcinogen increased EGFR expression and phosphoryrosine levels, those findings displayed in Fig. 4 demonstrate that RA opposed these effects at least partly through regulation of cyclin D1, a target common to the EGFR and retinoid signaling pathways.

Mechanisms involved in the retinoid regulation of EGFR in human bronchial epithelial cells were next pursued. Prior work revealed that cyclin D1 proteolysis was evident in RA-treated human bronchial epithelial cells even when repression of cyclin D1 mRNA was undetected (10). This occurred via a posttranscriptional mechanism that is activated by retinoid treatment and caused repressed expression of cyclin D1 protein (10, 11). Ubiquitin-dependent degradation of cyclin D1 was triggered by specific retinoids (10, 11, 21). This led to the view (11) that cyclin D1 is a candidate intermediate marker of retinoid chemoprevention response in the bronchial epithelium. The present study examined whether cyclin D1 was a chemoprevention target of the EGFR or the retinoid signaling pathways.

Consistent with prior work (10, 11), transfection into BEAS-2B cells of a reporter plasmid containing the human cyclin D1 promoter did not lead to an appreciable change in the transcriptional activity of this reporter plasmid, despite RA treatment, as shown in Fig. 5A. In contrast, activity of the EGFR reporter plasmid that contains an RA responsive motif (22) was significantly repressed by RA treatment of BEAS-2B cells, as depicted in Fig. 5A. As expected from the results of these cyclin D1 and EGFR reporter plasmid experiments, Northern analysis confirmed that RA treatment repressed EGFR mRNA expression, as shown in Fig. 5B. These findings were extended by showing that repressed expression of EGFR protein follows RA treatment of BEAS-2B cells (Fig. 5C). These findings indicate how transcriptional mechanisms are involved in the retinoid regulation of EGFR expression, whereas posttranscriptional mechanisms are activated in the retinoid repression of cyclin D1 protein.

Discussion

This study examined unique human bronchial epithelial cell lines as models for study of pathways involved in carcinogenic transformation as well as chemoprevention mechanisms in the lung. Cell lines studied included NNK-transformed BEAS-2B cells and BEAS-2B cells that were treated with RA and NNK (designated BEAS-2BNNK RA) in which transformation had been prevented (9), as summarized in Fig. 1. EGFR alterations were sought in these cellular models because aberrant EGFR expression is frequent in bronchial preneoplasia as well as in overt NSCLC (1–8). The findings presented in this study confirmed the value of these cellular models because aberrant expression of EGFR was detected as a consequence of NNK transformation. Notably, RA treatment prevented this transfor-
mation (9) as well as the observed increase in EGFR expression and EGFR-associated phosphotyrosine levels detected in BEAS-2B_NNK relative to BEAS-2B_NNK_RA cells, as shown in Figs. 1 and 2. The findings presented here build on prior work that highlighted cyclin D1 as a candidate intermediate marker of retinoid response in the bronchial epithelium (9–11) by showing that EGF-treatment stimulated mitogenesis of human bronchial epithelial cells and induced cyclin D1 expression. Notably, RA treatment opposed both of these actions. These and other findings presented in this study provided evidence for the EGFR as a target for lung cancer prevention.

Acute effects of RA on EGFR expression were studied. Acute RA treatment was shown to repress EGFR expression through transcriptional mechanisms, as depicted in Fig. 5. In contrast, acute changes in cyclin D1 mRNA expression (10) and cyclin D1 transcriptional activation (Fig. 5) were not observed after RA treatment. These findings are consistent with different mechanisms engaged in the retinoid regulation of EGFR and cyclin D1. Transcriptional mechanisms are activated in the

Fig. 4  EGF-induction of cyclin D1 expression and human bronchial epithelial cell growth. RA treatment opposed these effects. A, BEAS-2B cells were cultured in growth factor-free medium for 6 days (designated as time = 0), and cells were treated with EGF for 7, 15, or 30 additional hours, after which immunoblot analysis was performed for cyclin D1 expression. Cyclin D1 protein levels were induced by EGF treatment by 7 h and remained elevated for the 30 h of EGF treatment. In contrast, when RA was added for the last 6 h of EGF treatment, this EGF-dependent induction of cyclin D1 was blocked. In B, EGF treatment induced the mitogenesis of BEAS-2B cells. RA treatment prevented this growth stimulation. Proliferation assays were performed using thymidine incorporation, as described previously (10, 11). The proliferation assay depicted in this figure represented BEAS-2B cells treated under identical conditions as displayed in A. Compared with controls, EGF treatment induced BEAS-2B cell growth, but this was prevented by RA treatment. This experiment revealed that cyclin D1 was induced by EGF treatment, but this induction was repressed by RA treatment. 

Fig. 5 Different retinoid mechanisms regulate EGFR and cyclin D1 in human bronchial epithelial cells. In A, different mechanisms were activated in the repression of EGFR and cyclin D1 after RA treatment. Twenty-four h of RA (4 μM) treatment led to a significant reduction of EGFR but not of cyclin D1 reporter activity in BEAS-2B cells. For these cyclin D1 and EGFR reporter experiments, transcriptional activities were normalized to results obtained with a cotransfected β-galactosidase reporter plasmid. For the cyclin D1 reporter plasmid (D1pro-1749) experiments (18), luciferase activity was expressed as relative light units. For the EGFR reporter plasmid (pERCAT2) experiments (19), chloramphenicol acetyl transferase (CAT) activity was measured. The displayed findings were the results of at least three independent experiments, each performed in duplicate. Ps were obtained using a two-sided Fisher’s exact test. These assays were performed as described in “Materials and Methods.” In B, Northern analysis confirmed that acute RA (4 μM) treatment repressed EGFR expression in BEAS-2B cells. For these experiments, β-actin served as a loading control. +, treatment with 4 μM RA; −, treatment without 4 μM RA. In C, immunoblot analysis confirmed that RA (4 μM) treatment repressed EGFR expression in BEAS-2B cells. Actin expression served as a loading control. +, treatment with 4 μM RA; −, treatment without 4 μM RA. This assay was performed as described in “Materials and Methods.”
repressed expression of EGFR after RA treatment, whereas posttranscriptional mechanisms mediated the retinoid repression of cyclin D1 expression. These findings are consistent with results obtained after retinoid treatment of head and neck cancer cells (23). Thus, at least two independent mechanisms were activated by RA treatment to repress cyclin D1. One involved the posttranscriptional repression of cyclin D1 through the proteasome-dependent proteolysis of cyclin D1. The other repressed EGFR transcription and led to a decrease in EGFR mRNA expression. This reduction in EGFR expression blunted mitogenic response to EGF that would then reduce cyclin D1 expression. These findings are consistent with recent evidence for EGFR-dependent activation of the nuclear factor κB (NF-κB) that could affect cyclin D1 and cell cycle progression (24).

Acute effects of NNK on EGFR expression were examined to establish whether the observed increase in EGFR expression directly followed NNK exposure. The findings displayed in Fig. 3 indicate that an induction of EGFR did not occur immediately after acute NNK treatment of human bronchial epithelial cells. In contrast, there was a prominent induction of p53-associated DNA damage or oxidative stress mRNAs. This indicated that the observed increase in EGFR expression after carcinogen exposure was attributable to the outgrowth of EGFR overexpressing clones that arose despite a cellular response to repair genomic DNA damage caused by NNK. This could represent an adaptive response to carcinogen exposure that resulted in transformation of these bronchial epithelial cells. These findings indicated how RA selects against outgrowth of EGFR-overexpressing bronchial epithelial cells evident after carcinogenic damage. Although RA suppressed outgrowth of these EGFR-overexpressing human bronchial epithelial cells, RA may not be the optimal retinoid or class of therapeutic agent useful in this chemoprevention. Perhaps these cellular models would help identify the optimal chemopreventive agent used alone or as part of a combination regimen for lung cancer prevention.

These findings have clinical implications. EGFR is often overexpressed in subsets of NSCLC and in bronchial preneoplasia (1–8). Although identifying the precise mechanism(s) responsible for EGFR overexpression in carcinogen-exposed human bronchial epithelial cells is the subject of future work, what is apparent from the studies reported here is that the EGFR is a candidate target for lung cancer prevention. Targeting EGFR in clinical chemoprevention could be achieved by using antibodies that prevent ligand-dependent activation of the EGFR (1, 25), toxin-conjugated antibodies that target EGFR (26) or by using small molecules that inhibit the EGFR-specific tyrosine-kinase activity (15). A regimen to consider in clinical lung cancer prevention would combine retinoid treatment with an anti-EGFR agent to be prescribed at a lower dosage than typically used when these are administered as single agents. At least for those retinoids that broadly activate the retinoid nuclear receptors, chronic treatment is difficult to achieve because of vitamin A toxicities, as reviewed previously (27).

In summary, this study demonstrates that exposure of human bronchial epithelial cells to NNK transformed these cells and activated the EGFR. These findings are consistent with observations made relating EGFR expression to a carcinogen-induced bronchial epithelial xenograft model (28) and to studies of bronchial preneoplasia in lung cancer patients (8). Retinoid chemoprevention blocked both transformation of these cells (9) and an increase in EGFR expression in NNK-exposed human bronchial epithelial cells. NNK treatment caused a prominent induction of p53-associated DNA damage and oxidative stress species before an observed increase in EGFR expression. This indicated how RA suppressed the outgrowth of EGFR-overexpressing bronchial epithelial cells that were provided a growth advantage during this transformation process. Whether RA is the optimal chemopreventive agent useful in this cell context is the subject of future work. Notably, cyclin D1 was identified as a common target of the EGFR and retinoid signaling pathways, but different mechanisms were activated in the observed regulation of cyclin D1. Taken together, these findings extend prior work by showing that NNK exposure of human bronchial epithelial cells led to increased activity and expression of EGFR. This underscores the thought that EGFR is an attractive target for lung cancer chemoprevention. Administration of a retinoid with an anti-EGFR agent may yield therapeutic benefits in lung cancer prevention.

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References


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