Q14 HAMの治療法は？

HAMの経過は個人差が大きく、発病から数年で歩けなくなる重症例から、数十年経過しても歩行可能な軽症例まで、様々な経過をたどります。髄液検査で脊髄での炎症の程度を調べることにより、病気の進行をある程度予測することができるので、それぞれの進行度に応じた治療を行うことができます。

現在、HAMの治療法として有効性が認められているのは、脊髄で起きている炎症を抑える効果のある、ステロイド療法とインターフェロン注射療法です。これらの治療は、一時的な症状の改善や症状の進行を抑制するもので、完治させることができる治療法ではありません。

ただし、早いうちに治療を開始することで、病気の進行を最小限にとどめることができるので、できるだけ早く治療を始めることが重要です。

その他、足のしびれ、痛み、つっぱり感、便秘や排尿障害などの症状に対する薬物治療や、足のつっぱりを和らげたり筋力を維持するためのリハビリテーションも行われています。

詳しくは、難病情報センターのホームページで見ることができます。
http://www.nanbyou.or.jp/sikkan/128.htm

Q15 HU とは？

HU とは、HTLV-1 関連ぶどう膜炎（HTLV-1 associated uveitis）の略で、HTLV-1 感染が原因となって眼ぶどう膜に炎症が起こる病気です。ぶどう膜炎はHTLV-1 以外のウイルスや細菌、真菌、寄生虫などによっても起こる病気ですので、HTLV-1 はぶどう膜炎のたくさんある原因のうちの１つとなります。

症状は他の原因によって起こるぶどう膜炎と同じで、HU 特有の症状はありませんので、専門的な検査を行って総合的に診断する必要があります。

HU はキャリアの約0.1%に認められ、女性が男性の約２倍多く、特にバセドウ病の既往がある方に発症しやすいことが知られています。

Q16 HU の初期症状は？

HU の初期症状として以下の項目があげられます。

・眼の前に虫やゴミが飛んでいるようにみえる（飛蚊症）
・かすんで見える（霧視）
・眼の充血
・視力の低下

キャリアの方で上記のような症状が片眼もしくは両眼に急に起こった場合は、すみやかに医療機関を受診してください。診療科は眼科をおすすめします。

また、受診する場合には

・自分がキャリアであること
・いつから上記の症状があるか
・上記の症状の程度はどのくらいか

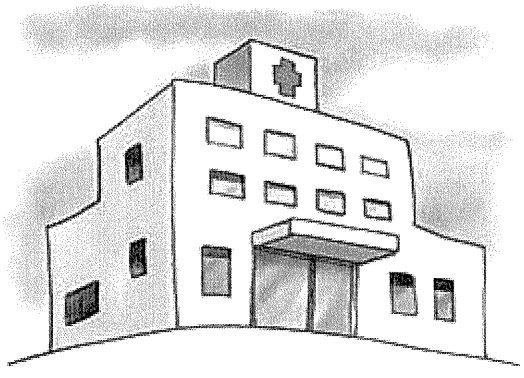
をきちんと医師に伝えてください。

Q17 HUの治療法は？

HUには副腎皮質ホルモン薬（ステロイド薬）がよく効きますので、点眼あるいは内服で治療します。およそ1～2ヶ月の治療でほとんどの方が治癒します。

ただし、約半数の方でHUが再発しますが、その場合には最初と同じように治療します。再発する頻度は1年に数回～数年に1回など、個人差がありますが、再発するたびにきちんと治療をすることで、長期的に視力を良好に保つことができます。

いずれの場合にも早期に治療を開始することが大切です。ぶどう膜炎を疑う症状（Q16参照）がある場合には、すみやかに医療機関を受診してください。



Q18 治療に対する医療費の助成は？

現在、ATLやHAM、HUに対する公的な治療費の助成はありません。

ただし、入院治療などによる自己負担が高額になった場合は、高額療養費制度により一部医療費の補助を受けられる場合がありますので、加入している健康保険の窓口に相談してください。また、1年間の自己負担が一定額を超えた場合には、確定申告によって所得税の医療費控除を受けられますので、お住まいの税務署に相談してください。

HAMの患者さんは、交付申請することで身体障害者手帳が交付される場合があります。その場合には、障害の程度に応じて身体障害者福祉制度による各種サービスを受けることができますので、お住まいの役所の福祉窓口に相談してください。

また、年金に加入している方で、障害により労働が不可能で日常生活に支障をきたしている場合には、障害年金の受給が可能な場合があります。くわしくは、加入している年金の窓口に相談してください。

Q19 患者会の活動は？

現在、ATLやHAMの患者さんが中心となって、全国に患者会が組織されています。HTLV-1に関する情報提供を行ったり、定期的に会合を開き、患者さんやご家族の悩みを語り合う場にもなっています。

また最近、患者や家族、医師などが協力し合い、HTLV-1の感染防止や正しい知識の普及を求めたNPO法人としての活動も行われています。この活動がきっかけとなって、平成21年度からHAMが厚生労働省難病対策疾患の指定を受けました。また、平成22年度からは、国がHTLV-1の総合対策を推進していくことになりました。

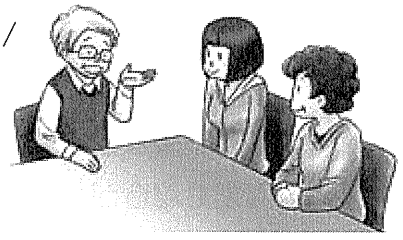
患者会の情報については以下のホームページをご覧ください。

【NPO法人「日本からHTLVウイルスをなくす会」】
http://www.minc.ne.jp/~nakusukai/

【NPO法人「はむるの会」】
http://www.hamuru.com/

【HAM患者会「アトムの会」】
http://www.minc.ne.jp/~nakusukai/index.atomu.htm

【長崎・佐賀HAM患者会ひまわり】
http://hamnagasaki.web.fc2.com/index.html



Q20 HTLV-1 に関する最新情報は？

HTLV-1 に関する最新情報は以下のホームページをご覧ください。

【厚生労働省「HTLV-1 について」】

http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou19/htlv-1.html

【国立感染症研究所「HTLV-1」】

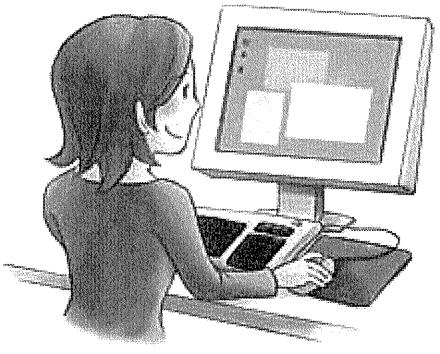
http://www.nih.go.jp/niid/HTLV-1/

【JSPFAD - HTLV-1 感染者コホート共同研究班 - 】

http://www.htlv1.org/

【HTLV-1 情報センター】

http://www.htlv1joho.org/



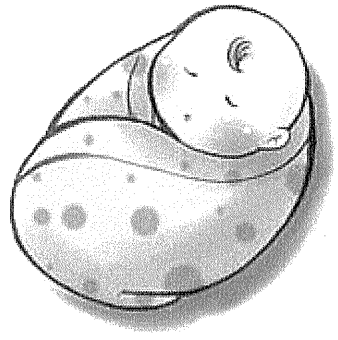
おわりに

一人で悩んでいてもHTLV-1感染の問題は解決しません。いまのところ、ATLやHAM、HUの発症を防ぐ有効な方法はありませんが、早期に治療を開始することで、発症後の生活を大きく改善することができます。

「病気かな？」と思ったら、迷わずに医療機関を受診してください。そしてHTLV-1に感染していることがわかっている方は、自分がキャリアであることをきちんと医師に伝えてください。そうすることで、すみやかに最善の治療を始めることができます。あなたの一言が、あなたの今後の生活を大きく変えることにつながるのです。

また、一部の医療機関ではキャリア外来を行っています。一人で不安を抱えながら生活を送るのではなく、定期的に検査をするなどして、医師と一緒に経過を観察していくこともできます。

少しでも疑問や不安がある場合は、お住まいの保健センターに相談してください。相談窓口では、HTLV-1感染に詳しい医師や、専門外来を行っている医療機関を紹介することもできます。まずは相談して、あなたが安心して生活を送ることができる方法を見つけてください。



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平成22年度厚生労働科学研究費補助金研究事業

難治性疾患克服研究事業
「重症度別治療指針作成に資するHAMの新規バイオマーカー同定と病因細胞を標的とする新規治療法の開発」
研究代表者　出雲 周二

第3次対がん総合戦略研究事業
「成人T細胞白血病のがん幹細胞の同定とそれを標的とした革新的予防・診断・治療法の確立」
研究代表者　渡邉 俊樹

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「成人T細胞白血病リンパ腫に対するインターフェロンαとジドブジン併用療法の有用性の検証」
研究代表者　塚崎 邦弘

「成人T細胞性白血病(ATL)の根治を目指した細胞療法の確立およびそのHTLV-1抑制メカニズムの解明に関する研究」
研究代表者　鵜池 直邦

平成22年度 初版

III. 研究成果の刊行に関する一覧表

雑誌

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制作物　（「HTLV-1総合対策」の一環として作成）

1. ウェブサイト「HTLV-1情報サービス」（http://www.htlv1joho.org/）

2. 小冊子「成人T細胞白血病の治療を受ける患者さん・ご家族へ
　　－　患者さんやご家族が納得した治療を受けていただくために」

3. 小冊子「HTLV-1キャリアのみなさまへ　－　よくわかる詳しくわかるHTLV-1」

IV. 研究成果の刊行物・別刷

Polycomb-Mediated Loss of miR-31 Activates NIK-Dependent NF-κB Pathway in Adult T Cell Leukemia and Other Cancers

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DOI 10.1016/j.ccr.2011.12.015

SUMMARY

Constitutive NF-κB activation has causative roles in adult T cell leukemia (ATL) caused by HTLV-1 and other cancers. Here, we report a pathway involving Polycomb-mediated miRNA silencing and NF-κB activation. We determine the miRNA signatures and reveal miR-31 loss in primary ATL cells. MiR-31 negatively regulates the noncanonical NF-κB pathway by targeting NF-κB inducing kinase (NIK). Loss of miR-31 therefore triggers oncogenic signaling. In ATL cells, miR-31 level is epigenetically regulated, and aberrant upregulation of Polycomb proteins contribute to miR-31 downregulation in an epigenetic fashion, leading to activation of NF-κB and apoptosis resistance. Furthermore, this emerging circuit operates in other cancers and receptor-initiated NF-κB cascade. Our findings provide a perspective involving the epigenetic program, inflammatory responses, and oncogenic signaling.

INTRODUCTION

Adult T cell leukemia (ATL) is an aggressive T cell neoplasm with very poor prognosis (Yamaguchi and Watanabe, 2002). Human T cell leukemia virus type I (HTLV-I) is recognized as an etiological factor in T cell malignancy. Although mounting molecular evidence has contributed to our ability to cure several cancers and other diseases, the genetic background of ATL leukemogenesis is not yet fully understood. Thus, it is an urgent request to clarify the molecular mechanism of ATL development.

Constitutive activation of nuclear factor-κB (NF-κB) is observed in the ATL cell lines and primary isolated tumor cells from ATL patients, although the viral oncoprotein Tax, a powerful activator of NF-κB, is not expressed in these malignant cells (Hironaka et al., 2004; Watanabe et al., 2005). NF-κB activation aberrantly contributes to cell propagation and anti-apoptotic responses in ATL and other cancers (Prasad et al., 2010). In our previous study, inhibition of NF-κB activity with a specific inhibitor, DHMEQ, drastically impaired the levels of ATL cell growth and resistance to apoptosis (Watanabe et al., 2005), suggesting that the molecular background of aberrant NF-κB activation may give us potential therapeutic targets. A recent report provided a new readout that NF-κB-inducing kinase (NIK) has a causal role in tumor progression and the aggressive phenotypes of various cancers, including ATL (Saitoh et al., 2008). NIK plays a pivotal role in the noncanonical (alternative) NF-κB pathway as a crucial kinase in receptor-initiating signaling, including signaling from CD40, LTBR, and BAFFR.

Significance

Here, we propose a molecular perspective of the onset of oncogenic signaling. NIK overexpression is a major driving force for constitutive NF-κB activation in various types of cancers. Using ATL cells as a model of NF-κB-addiction, we identified miR-31 as a suppressor of NIK that is completely silenced in ATL cells. Furthermore, an oncogenic function of a subset of Polycomb is implicated in NF-κB signaling via miRNA regulation. This study introduces a fundamental link between the Polycomb-mediated epigenetic regulation and the NF-κB signaling, allowing us to attribute the constitutive activation of NF-κB to epigenetic reprogramming.

Several studies have recently implicated another functional significance of NIK protein in epithelial cell proliferation, inflammatory response, and oncogenic signaling (for review, see Thu and Richmond, 2010). Although the expression level of NIK is strictly maintained by proteasomal degradation in normal cells (Liao et al., 2004), increased level of NIK transcript are observed in some cancers, causing inappropriate accumulation of NIK protein without stimuli (Annunziata et al., 2007; Saitoh et al., 2008). Overexpression of NIK leads to aberrant phenotypes in several cell types; however, little is known about the abnormal accumulation of NIK in malignant cells.

Recent advances have led to deeper understanding of a new aspect of posttranscriptional gene regulation, i.e., regulation by a class of noncoding RNAs. MicroRNAs (miRNAs) are functional RNAs with 18–25 nt in length that contribute to a class of cellular functions by negatively controlling targeted gene expression via base-pairing to 3′ untranslated region (3′ UTR). A single miRNA regulates the expression of multiple genes, and the functions of miRNAs therefore need to be orchestrated for cellular homeostasis (Ventura and Jacks, 2009). In the context of cancer pathology, many studies have provided evidences that miRNAs can act as either oncogenes or tumor suppressors. Although the relationship between miRNA deregulation and oncogenes has been clarified in several cancer cells, there has been no integrated analysis of gene expression in ATL. Since miRNAs have important functions in living cells, miRNA expression needs to be tightly regulated. Our knowledge about the regulatory mechanisms of miRNA expression is very inadequate because research effort has focused mainly on the role of miRNAs, which remains one of the most intriguing questions. miRNA regulation involves multiple steps. miRNA maturation has been identified as an important step, and its deregulation leads to progression and development of cancer (Davis et al., 2008; Trabucchi et al., 2009). Genetic deletion in cancer cells has also been reported to account for specific miRNA defect (Varambally et al., 2008). In addition, miRNA expression seems to be epigenetically programmed. DNA methylation and histone modification are strong candidates for miRNA regulation and their abnormalities, therefore, have causal roles in cancer initiation, development, and progression. In particular, Polycomb group proteins have central functions in cellular development and regeneration by controlling histone methylation, especially at histone H3 Lys27 (H3K27), which induces chromatin compaction (Simon and Kingston, 2009). Recent studies have revealed that the amount of Polycomb family is closely associated with cancer phenotypes and malignancy in breast cancer, prostate cancer, and other neoplasms (Sparmann and van Lohuizen, 2006). However, the substantial status of Polycomb family and their epigenetic impact in ATL cells have not been documented. Furthermore, the general roles of Polycomb proteins in miRNA regulation are mostly unknown. As described above, since miRNAs are multifunctional molecules in gene regulation, it is of pivotal importance to clarify the miRNAs functions and their regulatory circuit in order to formulate therapeutic strategies.

In the present study, we first performed global miRNA and mRNA profilings of the ATL cells derived from patients to precisely define the significance of miRNA expressions and functions.

RESULTS

miRNA Expression Signature in Primary ATL Cells

To characterize the miRNA expression signature in the primary ATL cells, we first performed an miRNA expression microarray analysis. For results with physiological significance, we used total RNA prepared from clinical ATL samples (n = 40, Table S1 available online) and control CD4+ T cells from healthy donors (n = 22) aged 50–70 years. A strict threshold ($p < 1 \times 10^{-5}$) and two-dimensional hierarchical clustering analysis revealed 61 miRNAs that showed significantly altered levels of expression in ATL cells compared with those of control CD4+ T cells (Figure 1A). It is noteworthy that 59 miRNAs out of 61 (96.7%) showed decreased expression in the primary ATL cells. Among them, we identified miR-31 as one of the most profoundly repressed miRNAs in all ATL individuals (fold change, 0.00403; Figure 1B). miR-31 was recently reported as a tumor suppressor and/or metastasis-associated miRNA in metastatic breast cancer. However, the biological functions of miR-31 in lymphocytes have not been studied. We therefore focused on the biological significance and regulatory mechanisms of miR-31 expression in T cells as well as in solid cancers.

miR-31 Negatively Regulates NF-κB Signaling via NIK Expression

To study the functional significance of miR-31 loss, we attempted to identify the target genes of miR-31 using four computational algorithms. We also performed gene expression microarray analysis of the primary ATL cells (n = 52, Table S1) and normal CD4+ T cells (n = 21) in order to detect aberrations in gene expression. Selected putative target genes are known to be involved in cell-cycle regulation and T cell development (Table S2). To experimentally identify the target genes, we performed reporter-based screens as described below. Luciferase-3′ UTR reporter assays demonstrated a remarkable negative effect against upstream gene expression by the *MAP3K14* 3′ UTR sequence (Figure S1B), which is consistent with an initial cloning report (Malinin et al., 1997). MAP3K14, also known as NIK, has a central role in noncanonical NF-κB signaling by phosphorylation of IKKα. A previous report (Saitoh et al., 2008) and the present results (Table S2) show that NIK is overexpressed in ATL cells, leading to constitutive NF-κB activation. As shown in Figure 2A, treatment with a miR-31 inhibitor increased *NIK* 3′ UTR reporter activity, suggesting the involvement of endogenous miR-31 in NIK downregulation. A computational search predicted one site each of miR-31 and miR-31 antisense (miR-31*) binding sites in the *NIK* 3′ UTR (Figure 2B). To identify the regulatory sequence in 3′ UTR of *NIK*, we established additional two reporters with mutated sequence in each potential seed region (Figure 2C; Figure S1C). Mutant 1, which contains mutated sequence in the miR-31 seed region, partially canceled the negative effect of endogenous miR-31 (Figure S1D) and prevented the effect of Anti-miR-31 treatment (Figure 2D). On the contrary, our results suggest that miR-31* does not participate in NIK regulation. miR-31-mediated reporter regulation was also observed in T cell lines (Figure S1E). To confirm the results, we repeated the experiment to examine whether miR-31 could inhibit NIK expression through seed sequence. We made expression plasmid vectors carrying NIK, NIK-3′

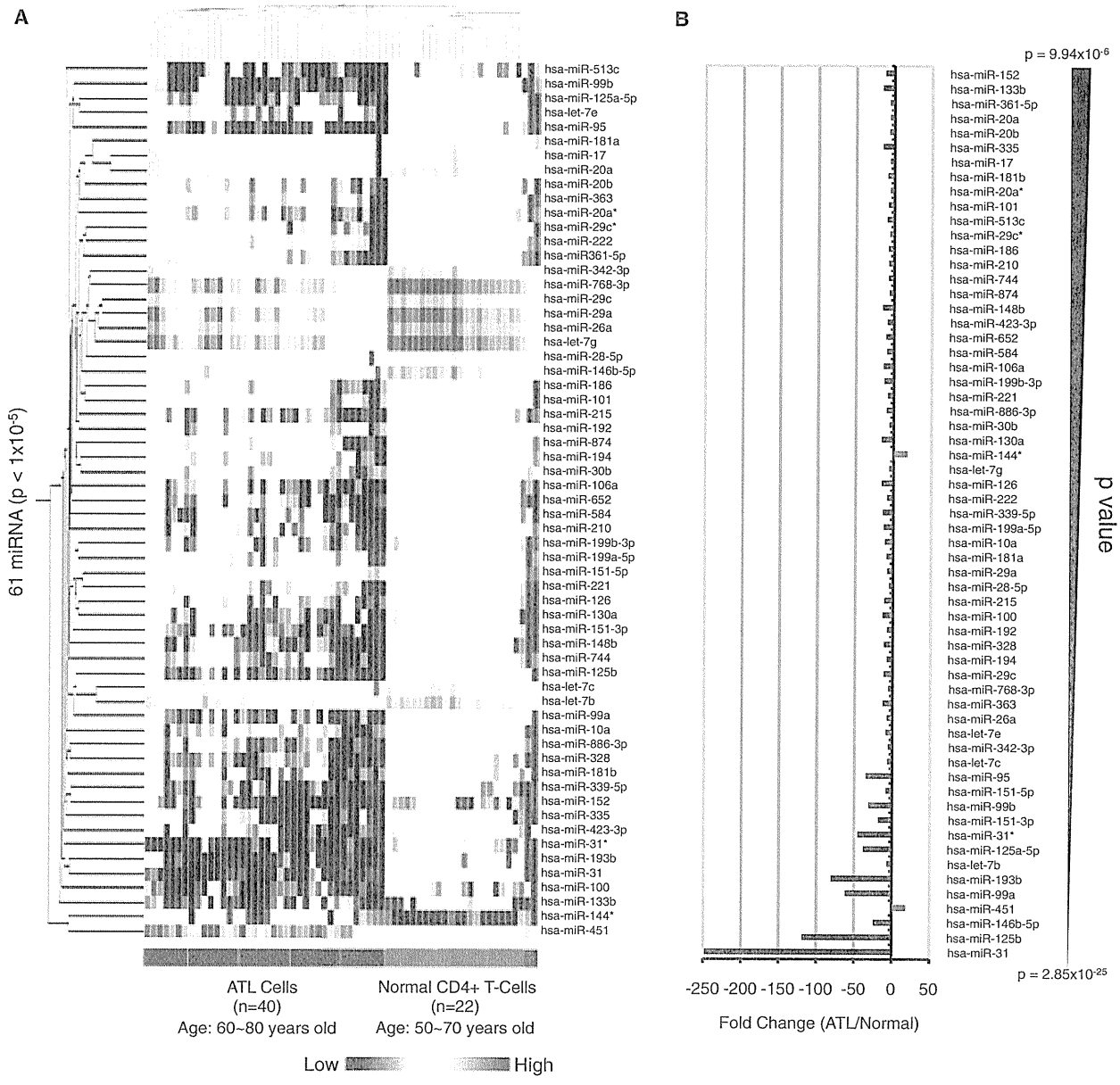

Figure 1. Global Profiling of Cellular miRNA on Primary ATL Cells

(A) Two-dimensional hierarchical clustering analysis and Pearson correlation as similarity measure on the miRNAs expressed at significantly different levels between the ATL (n = 40) and the control (n = 22) groups. Sixty-one miRNAs were identified ($p < 1 \times 10^{-5}$) and by filtering on more than 5-fold changes. A vertical branch shows the expression pattern of the selected miRNAs in each individual.

(B) Fold changes in the 61 miRNAs between ATL and Normal ($p < 10^{-5}$, fold change >5-fold). Selected miRNAs are arranged according to p values. See also Table S1.

UTRWT, or NIK-3′ UTRMu1 and tested their expressions in 293T cells. Results demonstrated that expression of NIK-3′ UTRWT was inhibited by simultaneous introduction of miR-31 (Figure 2E). miR-31 inhibition inversely rescued the NIK level, revealing that the cellular miR-31 level negatively affected that of the NIK protein through its 3′ UTR sequence. These lines of evidence collectively demonstrated that miR-31 recognizes and regulates *NIK* mRNA through specific binding to its 3′ UTR.

Transient introduction of the miR-31 precursor in TL-Om1 cells, which were established from an ATL patient, resulted in downregulation of NIK at the mRNA and protein levels, associated with downregulation of the phospho-IKKα/β level and NF-κB activity (Figures S1F and S1G). In contrast, miR-31 inhibition resulted in accumulation of NIK mRNA and protein in HeLa cells (Figure 2F). Manipulation of the miR-31 level clearly indicated that the miR-31 level negatively correlates with cellular