Oncrasin targets the JNK-NF-κB axis to sensitize glioma cells to TNFα-induced apoptosis

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Resistance of glioblastoma multiforme (GBM) to tumor necrosis factor (TNF) α-induced apoptosis have been attributed to increased nuclear factor-kappaB (NF-κB) activation. As we have previously reported that certain anticancer chemotherapeutics can sensitize glioma cells to TNFα-induced apoptosis by abrogating NF-κB activation, we investigated the potential of oncrasin in sensitizing glioma cells to TNFα-induced apoptosis. Oncrasin reduced glioma cell viability, inhibited TNFα-mediated NF-κB activation and sensitized cells to TNFα-induced apoptosis. Apoptosis was accompanied by elevated Fas and Fas-associated death domain (FADD) levels, increased caspase-8 activation and formation of death-inducing signaling complex (DISC). Oncrasin also (i) affected expression of cell cycle regulators, (ii) triggered DNA damage response, (iii) induced G0/M cell cycle arrest, (iv) decreased telomerase activity, (v) abrogated STAT3 activation and (vi) mediated extracellular release of high mobility group box 1 (HMGB1) along with its increased association with nucleosomes. Oncrasin-induced apoptosis did not involve mitochondria. Importantly, oncrasin increased c-jun N-terminal kinase (JNK) phosphorylation and pharmacological inhibition of JNK rescued oncrasin-induced apoptosis. JNK inhibition prevented oncrasin-induced decrease in TNFα-induced NF-κB activity and inhibition of NF-κB increased JNK phosphorylation in TNFα-treated cells. Oncrasin induced DISC formation and inhibited anchorage-independent growth of glioma cells in a JNK-dependent manner. By elucidating the existence of JNK-NF-κB cross-talk that regulates resistance to TNFα-induced apoptosis, this study has highlighted the importance of JNK in regulating viability of glioma cells.

Introduction

Constitutive nuclear factor-kappaB (NF-κB) activation in glioblastoma multiforme (GBM) tumors regulates expression of genes that promote their growth and survival (1), and inhibition of NF-κB activity induces glioma cell apoptosis (2). Being an important transcription factor associated with tumor promotion (3), NF-κB is regarded as a potential antiglioma target (4). Importantly, the resistance of glioma cells to tumor necrosis factor (TNF) α-mediated apoptosis (5) has been attributed to TNFα-induced NF-κB activation (6,7). We have reported that certain chemotherapeutic drugs can sensitize glioma cells to TNFα-induced apoptosis by abrogating NF-κB activation (8,9).

Aberrant activation of Ras occurs in GBM (10), and Ras is known to enhance NF-κB transcriptional activity (11,12). Besides Ras, STAT3 regulates constitutive NF-κB activation in tumors (13). STAT3 is constitutively expressed in GBM, and STAT3 inhibitors have shown promise as therapeutics for GBM (14). Importantly, STAT3 promotes Ras-dependent oncogenic transformation (15). Oncrasin induces apoptosis by affecting cellular targets whose function is dependent on K-Ras activation (16). NF-κB-mediated inhibition of c-jun N-terminal kinase (JNK) activation contributes to resistance to TNFα-induced apoptosis (17) and NF-κB negatively regulates TNFα-mediated JNK activation (18). Besides, JNK is a negative regulator of Ras-induced tumorigenesis (19). We have shown that histone deacetylase inhibitor induces glioma cell apoptosis in a Ras/JNK-dependent manner (20). Because oncrasin analog mediates apoptosis through simultaneous inhibition of STAT3 and increase of JNK activation (16), we evaluated the effect of oncrasin on glioma cell viability in the presence and absence of TNFα.

Our results suggest that in addition to inducing glioma cell apoptosis through increased JNK activation and STAT3 inhibition, oncrasin sensitizes glioma cells to TNFα-induced apoptosis in a JNK-dependent manner. Oncrasin-mediated abrogation of TNFα-induced NF-κB activity was concurrent with increased JNK activation. Importantly, increased activation of JNK upon inhibition of NF-κB activity and vice versa, highlighted the existence of a reciprocal interaction between JNK and NF-κB in glioma cells. Our findings suggest that TNFα might promote pro-survival advantage in glioma cells through regulation of this JNK-NF-κB axis.

Materials and methods

Cell culture and treatment

Glioblastoma cell lines A172, LN18 and T98G obtained from American Type Culture Collection were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Life Technologies-Invitrogen). On attaining semi-confluence, cells were switched to serum-free media, and after 6 h, cells were treated with different combinations of 50 ng/ml TNFα (R&D Systems) and oncrasin in the presence and absence of pan-caspase inhibitor (Calbiochem), caspase-8 inhibitor Z-IETD-FMK (Focris) or JNK (SP600125) inhibitor. All reagents were purchased from Sigma unless otherwise stated.

Determination of cell viability

Viability of glioma cells treated with different combinations of TNFα and oncrasin in the presence and absence of 50 µM pan-caspase inhibitor or 100 µM Z-IETD-FMK or 10 µM SP600125 was determined using the MTS (3-(4,5-dimethyldihiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium) assay (Promega) as described earlier (21).

Western blot analysis and immunoprecipitation

Protein from whole cell lysates and nuclear extracts from cells treated with different combinations of TNFα and oncrasin was separated on 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (22). Following antibodies were used: pATM (S1981) (Abcam), ATM (Abcam), high mobility group box 1 (HMGB1) (Abcam), cyclin D1 (Abcam), p27 (Abcam), caspase-8 (BD Pharmingen), Fas-associated death domain (FADD) (Millipore), Fas (Santa Cruz), p65 (Santa Cruz), IκBα (Santa Cruz), cyclin E (Santa Cruz), p21 (Santa Cruz), β-actin (Sigma), γH2AX (Ser 139) (Upstate), pJNK (Thr183/Tyr185), JNK, histone H3 and H4, pAK2 (Tyr1007/1008), JAK2 pSTAT3 (Tyr705), STAT3 and plkBα (Ser176/177). Antibodies were purchased from Cell Signaling unless otherwise mentioned. Immunoprecipitation was performed with Fas antibody to determine the association of Fas, FADD and caspase-8 in cells treated with different combinations of TNFα, oncrasin and JNK inhibitor as described (23).

Transfections and luciferase assay

For determining JNK levels in presence of dominant-negative IκBα, cells were transfected with 0.5 µg of DN-IκBα construct (Clontech and Takara) in the presence and absence of TNFα as described (22). In experiments with DN-constructions, control transfection using the appropriate empty vectors for each construct was employed. Reporter assay of cells transfected with NF-κB-luciferase constructs and treated with different combinations of TNFα, oncrasin and JNK inhibitor was performed using Lipofectamine 2000 (Life Technologies-Invitrogen) as described (22). NF-κB luciferase construct was cotransfected with Rluc construct.

Flow cytometric analysis of DNA content

Fluorescence-activated cell sorting (FACS) analysis of DNA content was performed to determine the effect of TNFα and oncrasin on glioma cell cycle progression as described (8). 10^6 cells per condition were fixed with 70% ethanol

Abbreviations: DISC, death-inducing signaling complex; FACS, fluorescence-activated cell sorting; FADD, Fas-associated death domain; GBCM, glioblastoma multiforme; HMGB1, high mobility group box 1; JNK, c-jun N-terminal kinase; NF-κB, nuclear factor-kappaB; TNF, tumor necrosis factor; TUNEL, Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling; SEM, standard error of the mean.
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and stored at −20°C. The fixed cells were resuspended in propium iodide solution (BD Biosciences) for 20 min at room temperature, and flow cytometric analysis was carried out using Cell Quest Program on FACS Calibur (Becton Dickinson).

Determination of mitochondrial-mediated apoptosis
Mitochondrial-mediated apoptosis was detected in cells treated with different combinations of TNFα, oncrasin and JNK inhibitor using MitoLight® mitochondrial apoptosis detection kit (Millipore) according to manufacturer’s instruction, and cells were examined by fluorescence microscopy. Colony formation in soft agar
The soft agar colony formation ability of glioma cells treated with oncrasin in the presence and absence of TNFα and JNK inhibitor was performed using CytoSelect™ 96-Well Cell Transformation Assay kit (Cell Biolabs), as described previously (24).

Statistical analysis
A value of $P < 0.05$ was considered significant.

Results
Oncrasin sensitizes glioma cells to TNFα-induced apoptosis
Treatment with oncrasin decreased glioma cell viability in a dose-dependent manner. Treatment with 20 µM oncrasin for 12 h resulted in
~20–30% reduction in cell viability. In cells treated with 40 and 50 µM concentration of oncrasin, the viability was similar to that observed at 20 µM of oncrasin. Although oncrasin had no significant effect on the viability of U87MG cells at lower concentration, the viability was significantly reduced at 50 µM concentration (Figure 1a). We, therefore, chose 20 µM dose of oncrasin for further characterization of its effect on glioma cell death. Combination of some anticancer chemotherapeutic drugs and TNFα has been reported to synergistically kill certain resistant tumor cells (25). As we have previously reported that casein kinase 2 inhibitors sensitize glioma cells to TNFα-induced apoptosis (9), we investigated whether oncrasin could induce apoptosis in TNFα-resistant glioma cells. Though glioma cell lines tested were resistant to TNFα-induced apoptosis, oncrasin sensitized glioma cells to TNFα-induced apoptosis. A ~35–40% decrease in cell viability was observed upon treatment of glioma cells with a combination of TNFα and oncrasin (Figure 1b). To further confirm oncrasin-mediated apoptotic death, Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay was performed (Figure 1c). The ~20–30% increase in TUNEL-positive cells observed upon oncrasin treatment was further elevated to ~35–50% when oncrasin was coadministered with TNFα (Figure 1c).

**Oncrasin decreases hTERT activity**

Telomerase inhibition is associated with apoptosis in cancers (26). Because telomerase inhibition is considered a promising antglioma therapeutic approach (27), we investigated telomerase activity of oncrasin-treated glioma cells in the presence and absence of TNFα. Although TNFα had no significant effect on telomerase activity, oncrasin abrogated telomerase activity by ~30–50%. However, in the presence of both oncrasin and TNFα, there was a 50–60% decrease in telomerase activity (Figure 1d).

**Oncrasin mediates HMGB1 release**

High mobility group box 1 (HMGB1), an architectural chromatin protein primarily associated with transcription regulation, is released into extracellular milieu in apoptotic cells. Glioma cells release HMGB1 upon cell death induced by cytotoxic agents (28). Because HMGB1 release is associated with pro-apoptotic killing in glioma cells (28), we determined the levels of secreted HMGB1 in glioma cells treated with oncrasin. HMGB1 was not observed in the culture media of either oncrasin or TNFα-treated cells. However, treatment with combination of oncrasin and TNFα resulted in a dramatic increase in HMGB1 release (Figure 1e). HMGB1 cofractionates with nucleosomes from apoptotic cells (29). Immunoprecipitation indicated increased interaction of HMGB1 with histones H3 and H4 in the media of cells treated with oncrasin and TNFα (Figure 1e).

**Oncrasin increases DISC formation upon TNFα treatment by enhancing association of Fas with FADD and elevating active caspase-8 expression**

Fas activation is associated with caspase-dependent apoptotic cell death as Fas stimulation results in the recruitment of adaptor molecule FADD (30). Interaction of Fas–FADD with procaspase-8 forms the death-inducing signaling complex (DISC), which stimulates caspase-8 activation that subsequently results in apoptosis through caspase-3 activation. We performed western blot analysis to determine the levels of FADD and Fas in cells treated with oncrasin in the presence and absence of TNFα. Although Fas and FADD levels remained unaffected upon TNFα treatment, an increase in Fas and FADD expression was observed in cells treated with oncrasin either alone or in combination with TNFα (Figure 2a). Although the expression of active caspase-8 was comparable between control, TNFα- and oncrasin-treated cells, a dramatic increase in active caspase-8 was observed upon TNFα and oncrasin treatment (Figure 2a).

**Fig. 2.** Oncrasin induces formation of DISC in TNFα-treated glioma cells. (a) Fas, FADD and caspase-8 expressions in glioma cells treated with oncrasin in the presence and absence of TNFα for 12h, as demonstrated by western blot analysis. A representative blot is shown from two independent experiments with identical results. Blots were reprobed for β-actin to establish equivalent loading. (b) Immunoprecipitation indicates increased DISC formation in oncrasin-treated glioma cells both in the presence and absence of TNFα. Lysate from cells treated with oncrasin or TNFα or both were immunoprecipitated with antibody to Fas.
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Fig. 3. Oncrasin affects genes associated with cell cycle regulation, DNA damage response and induces cell cycle arrest. Altered expression of molecules associated with (a) cell cycle progression and (b) DNA damage response in cells treated with oncrasin in the presence and absence of TNFα as demonstrated by western blot analysis. Figures (a and b) are representative blots shown from three independent experiments with identical results. Blots were reprobed with β-actin or C23 to establish equivalent loading. (c) Oncrasin induces G2/M phase cell cycle arrest in glioma cells. FACS analysis was performed on T98G cells treated with different combinations of TNFα and oncrasin. Inset indicates percentage of cells in G1, S and G2/M phase of the cell cycle. C, T, and O denote control, TNFα and oncrasin, respectively.

upon treatment with a combination of TNFα and oncrasin (Figure 2a). Coimmunoprecipitation was performed to determine whether elevated Fas, FADD and caspase-8 levels in TNFα− and oncrasin-treated cells were accompanied with increased DISC formation. Although the interaction of Fas, FADD and caspase-8 in TNFα-treated cells was comparable with the control, an increased interaction between them was observed in cells treated with either oncrasin or a combination of TNFα and oncrasin (Figure 2b). Oncrasin-induced apoptosis is accompanied by activation of caspase-8 in lung carcinoma cells (16). To confirm the involvement of caspase-8 in oncrasin-induced apoptosis, the viability of cells treated with TNFα and oncrasin in the presence and absence of caspase-8 inhibitor was determined. The cytotoxic effect of oncrasin on glioma cells was caspase dependent as treatment with Pan-caspase inhibitor as well as caspase-8 inhibitor rescued glioma cells from oncrasin-induced apoptosis both in the presence and absence of TNFα (Figure 2c).
Oncrasin affects molecules associated with cell cycle progression and DNA damage response

As oncrasin significantly inhibited the proliferation of glioma cells, we determined the expression of molecules associated with cell cycle progression in these cells. Although treatment with TNFα alone had no effect on the expression of cell cycle regulators p21, p27, cyclin D1 and E, oncrasin increased p21 and p27 levels and decreased cyclin D1 and E levels in glioma cells both in the presence and absence of TNFα (Figure 3a).

Oncrasin inhibits cell cycle progression

As oncrasin altered the expression of cell cycle regulators, FACS analysis was performed to determine cell cycle profile in cells treated with different combinations of oncrasin and TNFα. Although TNFα had no significant effect on cell cycle progression, oncrasin increased accumulation of cells at the G2/M phase (Figure 3c). A 8.21, 6.98, 15.23 and 19.13% cells at G2/M phase was observed in control, TNFα, oncrasin, and TNFα– and oncrasin-treated T98G cells, respectively. Similar trend was observed in A172 cells (data not shown).

Oncrasin induces increased DNA damage signaling response

HMGB1 is involved in DNA damage response and enhances DNA repair following damage (31). Because phosphorylation of histone H2AX on Ser 139 is a sensitive reporter of DNA double-strand breaks and as ATM is the main transducer of the double-strand break response (32), we investigated γH2AX and pATM expression in oncrasin-treated cells. Although treatment with TNFα or oncrasin alone had no effect on pATM level, an increase in pATM was observed upon treatment with a combination of both (Figure 3b). Although oncrasin elevated γH2AX levels in the cell lines in combination with TNFα (Figure 3b), it was capable of inducing γH2AX in T98G even in the absence of TNFα.

Oncrasin abrogates TNFα-induced NF-κB activation in glioma

As we have reported that TNFα-induced NF-κB transcriptional activity contributes to resistance to apoptosis in glioma cells (8), we determined whether downregulation of NF-κB activity in oncrasin-treated cells could have contributed to sensitivity to TNFα-induced death. IKKβ, which is the inhibitory subunit of NF-κB, is phosphorylated by IKKα/β, which
leads to IKKβ degradation and increased nuclear import of NF-κB (p65) (33). TNFα is known to increase phosphorylation of IKK, leading to decreased IKKβ levels and increased nuclear p65. Oncrasin mediated decrease in TNFα-induced pIkKαβ level in glioma cells (Figure 4a) was accompanied with increase in IkBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) and decrease in NF-κB levels (Figure 4a). Moreover, TNFα mediated increase in NF-κB luciferase activity was abrogated in the presence of oncrasin (Figure 4b).

**Pro-apoptotic effects of oncrasin are mediated by JNK pathway**

JNK is a member of the mitogen-activated protein kinase family that is activated in response to a variety of chemical stresses. Activation of JNK sensitizes glioma cells to apoptosis (34) by triggering apoptotic cascades (35). Mitogen-activated protein kinase kinase is involved in JNK activation, and the guanosine triphosphate-binding protein, Ras, plays a crucial role in MAP kinase activation (36). Besides, we have reported the involvement of JNK activation in glioma cell apoptosis (20). JNK suppresses Ras-stimulated tumor formation (19). Oncrasin has been shown to be effective against tumors bearing K-Ras mutations (16). As the antitumor activity of oncrasin analog is dependent on JNK activation (37), we investigated the phosphorylation status of JNK in oncrasin-treated cells. Treatment with oncrasin increased JNK activation in a time- and dose-dependent manner (Supplementary Figure 1, available at Carcinogenesis Online). Although TNFα negatively regulates TNFα-mediated JNK activation (18) and as oncrasin abrogated TNFα-induced NF-κB activation, the levels of pJNK levels in glioma cells treated with oncrasin alone or in combination with TNFα (Figure 4c). The pJNK levels in oncrasin-treated cells in the presence and absence of TNFα were comparable in LN18 cells. Despite pJNK levels being less in A172 and T98G cells treated with combination of oncrasin and TNFα as compared with that treated with oncrasin alone, the pJNK levels was still much higher than in untreated or TNFα-treated cells (Figure 4c). Because NF-κB antagonizes TNFα-mediated JNK activation (18), residual NF-κB level could have possibly prevented oncrasin from maximally activating JNK in the presence of TNFα in A172 and T98G cells.

We next investigated the role of elevated pJNK in inducing apoptosis in oncrasin-treated cells, by determining the viability of glioma cells treated with different combinations of TNFα and oncrasin in the presence and absence of JNK inhibitor SP600125. JNK inhibition significantly rescued glioma cells from apoptosis induced by oncrasin alone or in combination with TNFα (Figure 4d). Thus, increased JNK activation triggers the antiproliferative effect of oncrasin in the presence of TNFα.

**Non-involvement of mitochondria in oncrasin-triggered apoptosis**

Sustained JNK activation that occurs in the absence of NF-κB initiates apoptosis through induction of mitochondrial death pathway (38). We, therefore, investigated whether oncrasin-induced JNK activation triggers apoptosis through impairment of mitochondrial function. Accumulation of MitoLight red fluorescence, which is indicative of intact mitochondrial function, was comparable between oncrasin-treated and control cells (Supplementary Figure 2a, available at Carcinogenesis Online). Moreover, MitoLight accumulation was unaffected by JNK inhibition (Supplementary Figure 2a, available at Carcinogenesis Online). As JNK-mediated mitochondria-induced apoptosis involves antiapoptotic proteins Bcl-2 (39) and cytochrome c, we determined Bcl-2 and cytochrome c levels in oncrasin-treated cells. No change in the levels of both Bcl-2 and cytochrome c was observed upon oncrasin treatment both in the presence and absence of JNK inhibitors (Supplementary Figure 2b, available at Carcinogenesis Online).
Oncrasin mediated decrease in TNF-α-induced NF-κB activation is JNK dependent.

NF-κB pathway negatively modulates TNF-α-mediated JNK activation (18). Besides, suppressed NF-κB and sustained JNK activation sensitizes parthenolide to TNF-α-induced apoptosis in cancer cells (40). As oncrasin-mediated abrogation of TNF-α-induced NF-κB activation was accompanied by increased JNK activation, we questioned the involvement of JNK in NF-κB activation. The ability of oncrasin to downregulate TNF-α-induced NF-κB activation was abolished in the presence of JNK inhibitor (Figure 5a). Importantly, treatment with JNK inhibitor alone resulted in ~2–3-fold increase in TNF-α-induced NF-κB activity. This suggests that JNK downregulates
NF-κB activity, and that oncrasin mediated decrease in TNFα-induced NF-κB activation is JNK dependent.

**TNFα-induced NF-κB prevent JNK activation**

IKK negatively modulates JNK activity through induction of NF-κB target genes (18). As oncrasin-induced abrogation of TNFα-mediated NF-κB activation was concomitant with increased JNK activation, we investigated whether low JNK phosphorylation level in TNFα-treated cells was a consequence of heightened NF-κB activation. Interestingly, an increase in JNK phosphorylation was observed in TNFα-treated cells transfected with DN-IκB (Figure 5b). This together with the ability of JNK inhibitor to abrogate oncrasin-induced inhibition of TNFα-induced NF-κB activity suggests the existence of NF-κB-JNK axis that regulates the resistance of glioma cells to TNFα-induced apoptosis.

**JNK regulates DISC formation in glioma cells**

Activation of JNK and its dependent Fas-mediated death signaling sensitizes prostate cancer cells to etoposide-induced apoptosis (41). Moreover, JNK activation regulates FADD phosphorylation that subsequently sensitizes cancer cells to drug-induced apoptosis (42). Importantly, the role of Fas activation in JNK-mediated cell death in glioma cells is known (43). We, therefore, investigated whether oncrasin-induced JNK activation affects DISC formation. The increased interaction between DISC components—Fas, FADD and caspase-8 observed in oncrasin-treated cells both in the presence and absence of TNFα, was abrogated upon JNK inhibition (Figure 6a). Thus, oncrasin-induced DISC formation in glioma cells is JNK dependent (Figure 6a).

**Oncrasin mediated decrease in STAT3 phosphorylation is JNK independent**

The antitumor activity of oncrasin analog is mediated by STAT3 inhibition in addition to JNK activation (37). Heightened STAT3 activation plays a critical role in glioblastoma, and STAT3 inhibitors have shown promise as therapeutics for GBM (14). Also, targeting STAT3/JAK2 sensitizes glioma cell to apoptosis (44). We, therefore, investigated the effect of oncrasin on STAT3/JAK2 activation. Although treatment with TNFα had no effect on STAT3 or JAK2 activation, a decrease in pSTAT3 and pJAK2 levels was observed in glioma cells treated with oncrasin both in the presence and absence of TNFα. This decrease in STAT3 and JAK2 phosphorylation was independent of JNK as the decrease in STAT3 phosphorylation remained unaffected in the presence of JNK inhibitor (Figure 6b).

**Oncrasin mediated decrease in colony-forming ability of glioma cells is JNK dependent**

Although inhibition of JNK prevents epidermal growth factor-mediated anchorage-independent growth and protects glioma cells from cell death, JNK attenuates anchorage-independent growth in insulin-like growth factor-treated breast cancer cells (45). Because this dual function of JNK in terms of its ability to support anchorage-independent growth could be context dependent, we determined the ability of oncrasin to form colonies in soft agar. Although TNFα had no significant effect on colony-forming ability of glioma cells in soft agar, oncrasin reduced the anchorage-independent growth both in the presence and absence of TNFα. This decreased colony-forming ability of glioma cells in the presence of oncrasin was reverted to control levels, in the presence of JNK inhibitor (Figure 6c).

**Discussion**

We have reported the crucial involvement of TNFα-induced NF-κB activation in mediating resistance of glioma cells to TNFα-induced apoptosis (8,9). Here, we show that oncrasin sensitizes glioma cells to TNFα-induced apoptosis by inhibiting TNFα-induced NF-κB transcriptional activation. This decrease in NF-κB activation was concomitant with increased JNK activation, and pharmacological inhibition of JNK abrogated oncrasin-induced apoptotic cell death. Oncrasin triggered release of HMGB1 in TNFα-treated cells, which was detected in complex with nucleosomes. This ability of oncrasin to impair resistance to TNFα-induced apoptosis was accompanied with (i) arrest in G2/M phase of the cell cycle (ii) decreased STAT3/JAK2 activation and (iii) decreased telomerase activity (Figure 6d).

Although conflicting reports exist regarding the involvement of JNK in apoptosis, evidences indicate that sustained JNK activation triggers apoptosis (46). Although JNK activation in the absence of NF-κB initiates apoptosis through mitochondrial death pathway (38), oncrasin-mediated apoptosis did not involve mitochondria. Although JNK activation protects glioma cells from stress or chemotherapeutic-induced apoptosis (47), other studies have indicated that attenuation of TNFα-induced NF-κB activation leads to JNK-mediated glioma cell death (43). NF-κB activation is known to suppress TNFα-induced apoptosis through inhibition of caspase-8 and JNK activation (18). Oncrasin-mediated suppression of NF-κB activation was concurrent with increased JNK activation. This agrees with previous findings that absence of NF-κB-mediated inhibition of JNK activation contributes to TNFα-induced apoptosis (18). This negative effect of TNFα-induced NF-κB on oncrasin-induced JNK activation should have, therefore, reduced the apoptosis-inducing ability of oncrasin in the presence of TNFα. However, it is probable that other mechanisms, which sensitize cells to TNFα-induced apoptosis independent of JNK, contributed to the increased apoptosis in oncrasin and TNFα-treated cells. Besides, co-operativity between NF-κB and STAT3 regulates genes associated with tumor progression (48). It is possible that simultaneous disruption of both STAT3 (independent of JNK) and NF-κB activation could trigger greater cell death upon treatment with both oncrasin and TNFα.

NF-κB activation is known to protect leukemic eosiinophils from Fas-mediated apoptosis (49). Also, JNK-mediated Fas (41) and FADD (42)-dependent sensitization of cancer cells to drug-induced apoptosis is known. Increased JNK activation in response to oncrasin promoted DISC formation and caspase-8 activation leading to cell death (Figure 6d). In addition, oncrasin inhibited anchorage-independent growth of glioma cells in a JNK-dependent manner. Though JNK-mediated regulation of STAT3 activation is associated with doxorubicin resistance in cancer cell lines (50), oncrasin-mediated decrease in STAT3 activation was JNK independent. Overall, this study has highlighted the existence of an NF-κB-JNK axis in TNFα-treated glioma cells for the first time and has raised the possibility that chemotherapeutic regimen, which modulates NF-κB-JNK balance, might serve as an effective antiglioma therapeutic strategy.

**Supplementary material**

Supplementary Figures 1 and 2 can be found at http://carcin.oxfordjournals.org/

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**References**


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