TRAIL and Interferon-α Act Synergistically to Induce Renal Cell Carcinoma Apoptosis

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Abbreviations and Acronyms

c-FLIP = cellular FLIP
cIAP = cellular inhibitor of apoptosis
DAPI = 4,6-diamidino-2-phenylindole
DcR = decoy receptor
DD = death domain
DMSO = dimethyl sulfoxide
DR = DD containing receptor
ELISA = enzyme-linked immunosorbent assay
ERK = extracellular signal-regulated kinase
FLIP = Fas associated death domain-like interleukin-1β-converting enzyme-inhibitory protein
IFN = interferon
Mcl = myeloid leukemia cell differentiation protein
MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
PARP = poly(adenosine diphosphate-ribose) polymerase
PBS = phosphate buffered saline
PI = propidium iodine
PKC = protein kinase C
PUMA = p53 up-regulated modulator of apoptosis
RCC = renal cell carcinoma
Smac/DIABLO = second mitochondria derived activator of caspase/direct inhibitor of apoptosis binding protein with low pI
TNF = tumor necrosis factor
TRAIL = Apo2 ligand/tumor necrosis factor related apoptosis inducing ligand
VEGF = vascular endothelial growth factor
XIAP = X-linked inhibitor of apoptosis

Purpose: Despite modern targeted therapy metastatic renal cell carcinoma remains a deadly disease. Interferon-α (Calbiochem®) is currently used to treat this condition, mainly combined with the targeted anti-vascular endothelial growth factor antibody bevacizumab. TRAIL (Apo2 ligand/tumor necrosis factor related apoptosis inducing ligand) (Calbiochem) is a novel antineoplastic agent now in early phase clinical trials. Interferon-α and TRAIL can act synergistically to kill cancer cells but to our knowledge this has never been tested in the context of renal cell carcinoma. We hypothesized that TRAIL and interferon-α could synergistically induce apoptosis in renal cell carcinoma cells.

Materials and Methods: We treated renal cell carcinoma cell lines with recombinant TRAIL and/or interferon-α. Viability and apoptosis were assessed by MTS assay, flow cytometry and Western blot. Synergy was confirmed by isobologram. Interferon-α induced changes in renal cell carcinoma cell signaling were assessed by Western blot, flow cytometry and enzyme-linked immunosorbent assay.

Results: TRAIL and interferon-α acted synergistically to increase apoptotic cell death in renal cell carcinoma cells. Interferon-α treatment altered the ability of cells to activate extracellular signal-regulated kinase while inhibiting extracellular signal-regulated kinase with UO126 abrogated TRAIL and interferon-α apoptotic synergy. Interferon-α did not induce changes in TRAIL or death receptor expression, or change other known mediators of the intrinsic and extrinsic apoptotic cascade in the cells.

Conclusions: TRAIL plus interferon-α synergistically induces apoptosis in renal cell carcinoma cells, which is due at least in part to interferon-α mediated changes in extracellular signal-regulated kinase activation. TRAIL and interferon-α combination therapy may be a novel approach to advanced renal cell carcinoma that warrants further testing in vivo.

Key Words: kidney; carcinoma, renal cell; TNF-related apoptosis-inducing ligand; interferons; extracellular signal-regulated MAP kinases

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METASTATIC RCC, which is notoriously resistant to traditional cytotoxic chemotherapy, is treated with various forms of immunotherapy and targeted therapy. Immunotherapy includes IFNα and interleukin-2, which induce a response in 10% to 20% of cases, of which only a third are durable, complete responses.[1] Targeted therapies include small molecule tyrosine kinase inhibitors, monoclonal antibodies to vascular endothelial growth factor (bevacizumab) and inhibitors of mammalian target of rapamycin.[2] These targeted therapies have improved the outlook of advanced RCC but cure remains rare and novel forms of therapy for advanced RCC are being actively investigated.

Although IFNα, a type I IFN, can directly induce apoptosis in a number of cancer cell lines, including melanoma, multiple myeloma and ovarian cancer,[3,4] its mechanism of action for RCC has never been fully elucidated and it is thought to be mediated by immune system modulation. IFNα was 1 of the first therapies to show a survival advantage in RCC cases and it is less toxic than the alternative immunotherapy, high dose interleukin-2.[1] Thus, it was extensively used before the advent of targeted therapy. Currently IFNα use has significantly decreased, although it is still used in combination with bevacizumab.

TRAIL is a member of the TNF receptor superfamily that includes CD95 ligand (FasL) and TNFα. The usefulness of TRAIL as a therapeutic anticancer agent has been extensively investigated since all members of the TNF superfamily can induce apoptosis via the extrinsic pathway.[5,6] However, only TRAIL and not FasL or TNFα is relatively well tolerated when given systemically.[7,8] In xenograft models tumor regression was reported in response to systemic TRAIL while TRAIL based therapy is currently in early phase clinical trials.[9,10]

TRAIL initiates apoptosis by binding 1 of its cognate DRs, DR4 or DR5.[11] These receptors then interact with Fas associating DD containing protein adapter protein,[12,13] which aggregates to allow the death effecter domain of Fas associating DD containing protein to interact with the death effecter domain of initiator caspases 8 and 10. This leads to a proteolytic cascade, ultimately resulting in apoptosis and cell death. TRAIL can also bind to 2 receptors, DcR1 and DcR2, that function as DcRs and bind ligand but do not induce apoptosis because they lack a functional DD.

Synergy between TRAIL mediated killing and more traditional chemotherapy agents and/or radiation therapy has been noted in vitro and in vivo in a wide variety of malignancies, including RCC.[7,14,15] Some tumor cells resistant to either therapy alone undergo apoptosis in response to combination therapy.[14,15] IFNα can up-regulate TRAIL expression in various cell types[3,16] and IFNβ, another type I IFN, can sensitize cells to TRAIL induced apoptosis.[17] Thus, we tested the hypothesis that TRAIL and IFNα would act synergistically to induce RCC cell death.

MATERIALS AND METHODS

Cell Viability

We determined cell viability using the MTS method (Promega, Madison, Wisconsin) using the manufacturer protocol. Briefly, cells were seeded in 96-well culture plate, grown overnight and treated as indicated. MTS/phenazine methosulfate solution was added for 1 hour and absorbance was measured at 490 nm. All experiments were done in triplicate and results are shown as the mean ± SE.

Isobologram for Synergy

To determine whether combination treatment was additive or synergistic cells were exposed to varying doses of IFNα (250 to 4,000 IU/ml) for 72 hours, to TRAIL (62.5 to 1,000 ng/ml) for 24 hours or to a combination. Cell viability was determined using the MTS method. An additive or synergistic response was determined by isobologram with analysis based on the median effect principal. CalcuSyn software (Biosoft®) allows one to calculate the combination index, for the drug combination compared with that of each agent alone, including a combination index of 1—additive effect, greater than 1—antagonism, less than 1—synergy and less than 0.5—strong synergy.[18] A scoring system of +++++ (very strong synergy) to −−−−− (very strong antagonism) was used, as recommended by Chou and Talalay.[18] All experiments were done in triplicate and representative results are shown.

Western Blot

Cells were lysed in lysate buffer composed of 50 mM tris (pH 8.0), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid and 0.5% NP-40 with protease inhibitor solution (Roche, Mannheim, Germany). Protein concentration was determined using a protein assay (Bio-Rad®). Equal protein was loaded onto 10% to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, transferred to nitrocellulose or polyvinylidene difluoride membrane, blocked with 5% dry milk in tris buffered saline-Tween, incubated with primary antibody, washed, incubated with secondary horseradish peroxidase labeled antibody and washed. Bands were visualized using LumiGLO® Reagent and Peroxide.

Flow Cytometry

Cells were allowed to grow overnight, treated as indicated, trypsinized, resuspended in medium with 10% fetal bovine serum and washed twice with cold PBS. To determine DR expression cells were blocked with 1% bovine serum albumin in PBS, incubated with primary antibody, washed and resuspended in 1% bovine serum albumin in PBS with secondary antibody. Cells were washed again and resuspended in PBS. Counts were read using a FACSCalibur™ flow cytometer. For cell cycle analysis after washing cells were fixed in cold 70% EtOH, stained