NUC-7738, a novel ProTide transformation of 3′-deoxyadenosine, activates AMPK and kills renal cancer cells

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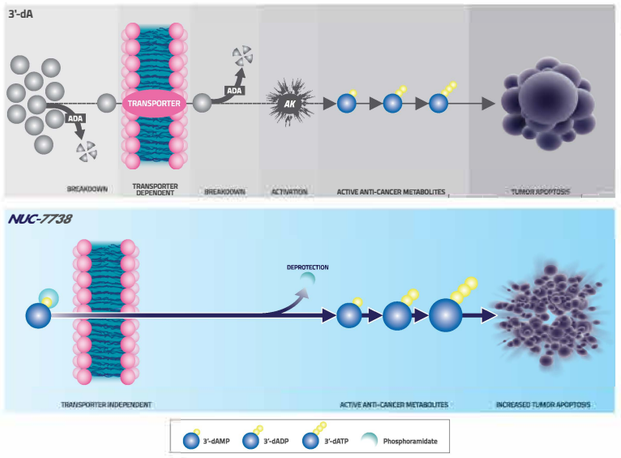
Background

- 3′-deoxyadenosine (3′-dA; cordycepin) is an adenosine derivative isolated from *Cordyceps sinensis*
- 3′-deoxyadenosine triphosphate (3′-dATP), the active anti-cancer metabolite of 3′-dA, causes cell death by inhibiting DNA and RNA synthesis, inducing apoptosis and activating AMPK[1,2]
- 3′-dA has not been successful in clinical studies due to cancer resistance mechanisms including:
  - Rapid enzymatic degradation by adenosine deaminase (ADA)
  - Cellular uptake dependent on nucleoside transporters (hENT1)
  - Rate limiting phosphorylation by adenosine kinase (AK) to generate the active metabolite (3′-dATP)

NUC-7738: A ProTide Transformation of 3′-dA

- Overcomes key 3′-dA resistance mechanisms:
  - Protected from breakdown by ADA
  - Cellular uptake independent of hENT1
  - 3′-dATP generation independent of enzymatic activation by AK
- *In vitro* data across various cancer cell lines demonstrate that NUC-7738 has substantially greater cytotoxicity (up to 185-times greater) than 3′-dA

NUC-7738 overcomes the key cancer resistance mechanisms of 3′dA



Renal cell carcinoma

- Renal cell carcinoma (RCC) is the most common type of kidney cancer[3]
  - Clear cell RCC (ccRCC) is the most common histological subtype (>70%)[4]
- Numerous metabolic pathways are affected in ccRCC[5]
  - Decreased AMPK activity and increased activation of mammalian target of rapamycin (mTOR) signaling have been implicated in tumor development and progression[6]
  - mTOR-targeted therapies have shown activity in the treatment of RCC[7]
- A hypoxic environment is a key feature in ccRCC and plays an important role in tumor resistance to anti-cancer therapies[8]

Methods

Tissue Micro Array Studies

- Tissue Micro Arrays (TMAs) for primary (*n=303* patients) and metastatic (n=169 patients) ccRCC obtained from SCOTRRCC cohort[9]
- Assessed by immunofluorescence using anti-AMPK, anti-pAMPK, anti-RCK+CA9

Cell Line Studies

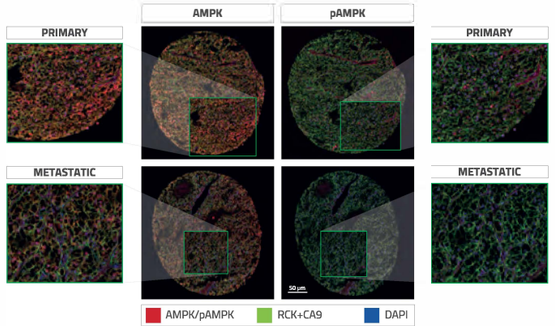
- Four ccRCC cell lines investigated; 786-O, 769-P, UMRC-2 and Caki-1
- Effect of hypoxia (0.5% O_2) on AMPK and pAMPK protein expression assessed by Western blot
- Cell viability assessed by sulforhodamine-B assay
- Effect of NUC-7738 on protein expression of pAMPK and p4E-BP1 (Thr37/46) under normoxic and hypoxic conditions assessed by Western blot and quantified using Licor Odyssey

Ex vivo Tissue Studies

- *Ex vivo* ccRCC tissue cut into 200 µm sections with Leica Vibratome and cultured in M199 media in 5% CO_2/air conditions
- Tissue exposed to 5-100 µM NUC-7738 for 24 hours before being assessed by immunofluorescence using anti-pAMPK and anti-cleaved caspase-3

Results

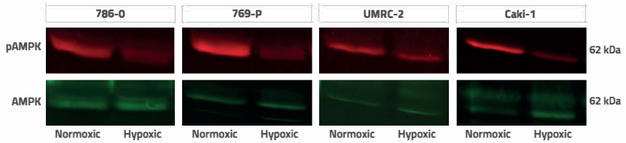
ccRCC tumors express abundant AMPK but very low levels of activated AMPK



TMA sections from primary and metastatic RCC

- AMPK protein expression uniformly high in primary and metastatic tumors
- In contrast, activated AMPK (pAMPK) undetectable or very low
- Where detected, activated AMPK was focal in expression with a high degree of inter- and intra-patient heterogeneity

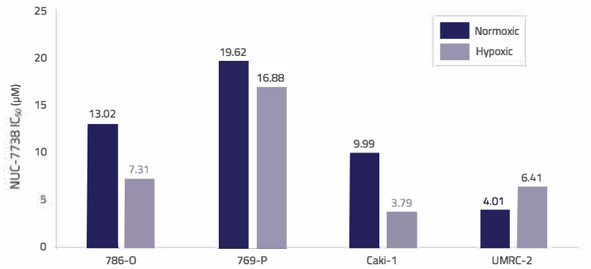
Under hypoxic conditions AMPK activation was reduced



Western blots of AMPK and activated AMPK in four ccRCC cell lines.

- AMPK was expressed in all four ccRCC cell lines under both normoxic and hypoxic conditions
- Hypoxia reduced the activation of AMPK in all ccRCC cell lines

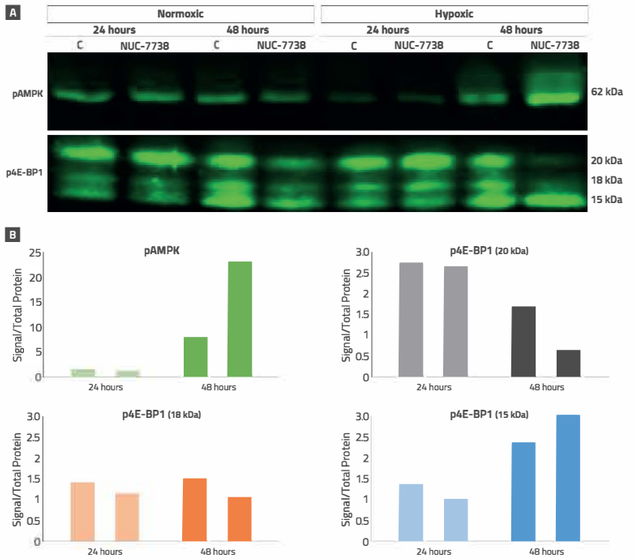
Cell proliferation is inhibited by NUC-7738 under normoxic and hypoxic conditions



IC_{50} values of NUC-7738 in normoxic and hypoxic conditions in four ccRCC cell lines.

- NUC-7738 retains anti-tumor activity in both normoxic and hypoxic conditions

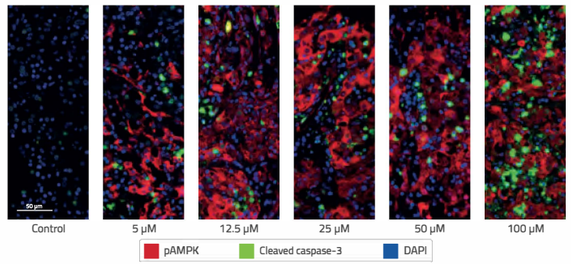
NUC-7738 increases AMPK activation and reduces 4E-BP1 phosphorylation in 786-O cells



A. *Western blot of activated AMPK and phosphorylated 4E-BP1 (surrogate for mTOR activity) in 786-O cells exposed to NUC-7738 in normoxic and hypoxic conditions.*

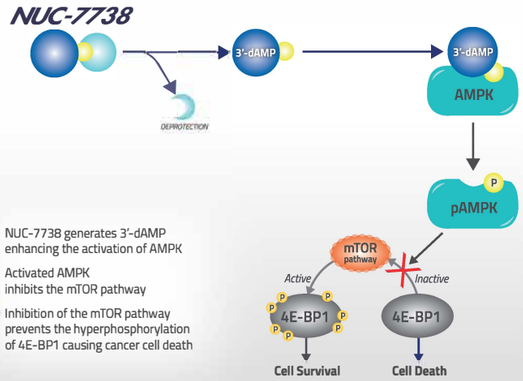
B. *Quantification of Western blot bands for activated AMPK and 4E-BP1 phosphorylation bands under hypoxic conditions.*

NUC-7738 increases AMPK activation and induces cell death in *ex vivo* ccRCC tissue



- AMPK was activated in the presence of NUC-7738 at doses ranging from 5 µM to 100 µM
- Cleaved caspase-3 expression (indicating cell death) increased with NUC-7738 concentration

3′-dAMP enhances activation of AMPK which inhibits mTOR pathway



- NUC-7738 generates 3′-dAMP enhancing the activation of AMPK
- Activated AMPK inhibits the mTOR pathway
- Inhibition of the mTOR pathway prevents the hyperphosphorylation of 4E-BP1 causing cancer cell death

Conclusion

- Activation of AMPK was low in both primary ccRCC tissue and cell lines grown under hypoxic conditions
- NUC-7738 caused activation of AMPK in ccRCC cell lines and in *ex vivo* ccRCC tissue
- NUC-7738 demonstrated anti-cancer activity in ccRCC cell lines in normoxic and hypoxic conditions and increased cell death in *ex vivo* ccRCC tissue
- NUC-7738 may also exert its anti-tumor activity through inhibition of the mTOR signaling pathway
- Renal cancer tissue typically has low expression of pAMPK, raising the prospect that AMPK modulation may offer a therapeutic option for ccRCC
- NUC-7738 is currently being investigated in a Phase I clinical study (NuTide:701) in patients with solid tumors and lymphoma
  - NuTide:701 will determine the RP2D and schedule of NUC-7738
  - Six patients have been dosed to date

ABBREVIATIONS: 3′dA: 3′-deoxyadenosine 3′-dATP: 3′-deoxyadenosinetriphosphate AMPK: 5′-adenosine monophosphate-activated protein kinase pAMPK: phospho-AMPK ADA: adenosine deaminase hENT1: human equilibrative nucleoside transporter 1 AK: adenosine kinase RCC: renal cell carcinoma ccRCC: clear cell RCC mTOR: mammalian targetof rapamycin TMA: tissue micro array p4E-BP1: phospho-eIF4E-binding protein RCK: DEAD-box helicase 6 CA9: carbonic anhydrase 9 DAPI: 4′,6-diamidino-2-phenylindole RP2D: recommended phase 2 dose LKB1: liver kinase B1 IC_{50}:half-maximal inhibitory concentration

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Data cut-off 7th October 2019. Data cleaning ongoing.