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Exchange Protein Directly Activated by cAMP (EPAC1) Protects Human Endothelial Cells from Tumor Necrosis Factor-α (TNF-α) Induced Cell Death

Anh Luu
Ohio Northern University

Abigail Carpenter
Ohio Northern University

Rosetta Tolley
Ohio Northern University

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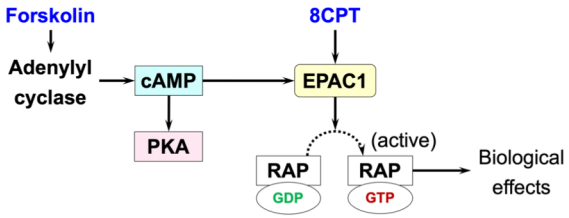
Anh Luu, Abigail Carpenter, Rosetta Tolley, and Mark Olah
Department of Pharmaceutical and Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University

INTRODUCTION

- **TNF-α** is a pro-inflammatory cytokine. TNF-α is elevated during inflammatory responses, such as ischemia/reperfusion injury, sepsis and COVID-19 infection. TNF-α:
  - Promotes the release of other cytokines such as IL-1, IL-6 and thromboxane A2.
  - Induces cellular oxidative stress.
  - Exacerbates tissue damage and injury.
  - Causes dysfunction and death of endothelial cells (EC).
- **EPAC1** (exchange factor directly activated by cAMP) is an intracellular cAMP sensor that activates Rap family of small G-proteins and stimulates a downstream signaling cascade. In EC, EPAC1 is involved in:
  - Vascular barrier function.
  - Angiogenesis and endothelial proliferation.



HYPOTHESIS

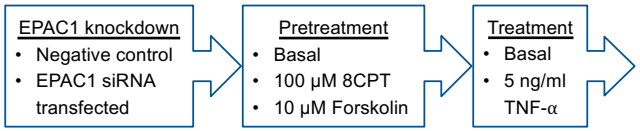
Elevation of cAMP and specific activation of EPAC1 in EC is protective against TNF-α-mediated apoptosis.

METHODOLOGY

- Experiments were performed in triplicate or quadruplicate.
- **Cell culture:** human microvascular EC (PromoCell) grown in EGM™-2 (Lonza).
- **EPAC1 knockdown** using silencing RNA (siRNA). Cells were transfected with 100 nM control siRNA or EPAC1 siRNA with Lipofectamine® RNAiMAX in Opti-MEM® Medium.
- **Pretreated** for 45 minutes with:
  - 100 μM **8-pCPT-2′-O-Me-cAMP** (**8CPT**, a direct, specific activator of EPAC1), obtained from Biolog.
  - Or 10 μM **Forskolin** (an adenylyl cyclase activator that increases intracellular cAMP level), obtained from Tocris.

METHODOLOGY

- **Treated** with 5 ng/ml **TNF-α** (Sigma) and maintained in EBM™-2 (Lonza) for 24 hours.
- **Cell viability** determined with MTT assay. Cells were exposed to 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Alfa Aesar) for 4 hours and then lysed with dimethyl sulfoxide (Fisher). Absorbance were read at 540 nm.
  - Absorbance data, representative of cell viability, was express as percentage relative to control group.
- **Cleaved caspase-3 levels** were determined with Western Blotting. Proteins were separated on 12% SDS-PAGE gels and transferred to nitrocellulose membranes, followed by blotting with cleaved caspase-3 antibodies (Cell Signaling). Chemiluminescent signals, which were proportional to relative protein levels, were detected by autoradiography.
  - Increased cleaved caspase-3 expression indicates apoptosis.



RESULTS

Pretreatment with 8CPT or Forskolin inhibits TNF-α-induced EC death

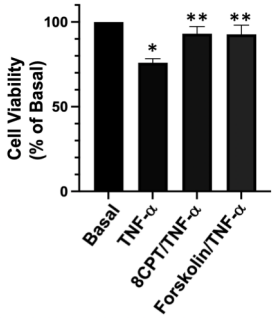


Figure 1. Relative to basal, TNF-α decreased cell viability to 76.0±2.4%. Pretreatment with 8CPT or Forskolin prior to TNF-α exposure increased viability to 93.1±4.3% and 92.7±5.4%, respectively. *p<0.01 relative to basal. **p<0.01 relative to TNF-α alone.

RESULTS

siRNA knockdown of EPAC1 abolishes the protective effect of 8CPT on TNF-α-induced death while the activity of Forskolin is unaffected

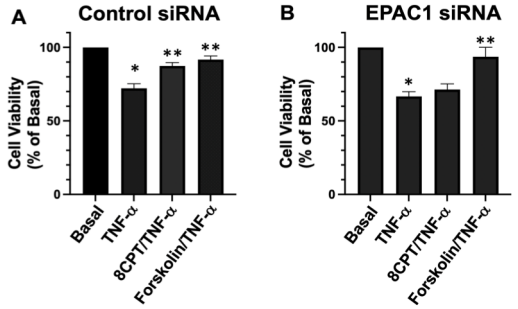


Figure 2. (A) In control-transfected cells, TNF-α decreased cell viability to 72.2±3.1%. Pretreatment with 8CPT or Forskolin prior to TNF-α exposure increased viability to 87.4±2.3% and 91.7±2.5%, respectively. **(B)** In EPAC1 siRNA-transfected cells, TNF-α decreased cell viability to 66.7±3.2%. In EC, pretreated with 8CPT or Forskolin prior to TNF-α exposure cell viability was 71.3±3.9% and 93.6±6.4%, respectively. For both panels, *p<0.01 relative to basal. **p<0.05 relative to TNF-α alone.

TNF-α increases cleaved caspase-3 levels and that effect is reduced by 8CPT or Forskolin pretreatment

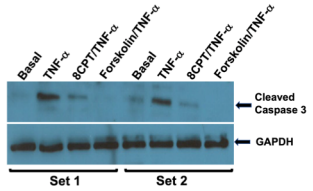


Figure 3. Relative to basal, TNF-α treatment elevated the expression of cleaved caspase-3, and pretreatment with 8CPT or Forskolin suppressed such increase. Two representative blots of an experiment repeated 4 times. GAPDH was used as a loading control.

CONCLUSIONS

- Elevation of cAMP and activation of EPAC1 suppressed TNF-α-driven endothelial cell apoptosis.
- The EPAC1 signaling axis is a potential therapeutic target for vascular protection in inflammation-driven injury.

ACKNOWLEDGEMENTS

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