

Research Proposal: Does SGK Block Cell Death by Controlling BAD?

We propose that serum- and glucocorticoid-induced kinase 1 (SGK) suppresses apoptosis through dual regulation of the BH3-only protein BAD: direct phosphorylation at Ser112 and Ser136, and an unanticipated increase in BAD expression.

Background and rationale

Evasion of apoptosis underlies resistance to cytotoxic and hormonal therapy. BAD, a BH3-only member of the Bcl-2 family, occupies the mitochondrial apoptotic switch: in its unphosphorylated state it neutralizes Bcl-xL and initiates cell death, whereas phosphorylation at Ser112 and Ser136 sequesters it in the cytosol with 14-3-3 proteins. SGK is a pro-survival kinase induced by glucocorticoids, androgens and serum, activated downstream of PDK1 and mTORC2, and overexpressed in prostate, breast and lung carcinomas. It shares the RxRxxS/T substrate motif with Akt, the kinase conventionally credited with BAD phosphorylation, yet its own apoptotic substrates remain undefined.

Preliminary data from this laboratory implicate SGK in BAD regulation at two levels. Purified constitutively active SGK phosphorylates recombinant BAD at Ser112 *in vitro*, and in cells elevates phosphorylation at both Ser112 and Ser136 ($p < 0.01$, $n = 4$). Conversely, siRNA-mediated SGK depletion in PC-3 prostate carcinoma cells reduces insulin-stimulated Ser112 phosphorylation ($p = 0.041$, $n = 3$) and cell survival ($p = 0.013$, $n = 3$). Unexpectedly, SGK also governs BAD abundance: active SGK increased BAD transcript approximately 10-fold, and dexamethasone-induced endogenous SGK raised BAD protein roughly 3-fold ($p = 0.039$, $n = 3$).

Specific objectives: These observations present an apparent paradox: a survival kinase that elevates a pro-apoptotic protein. We hypothesize that SGK expands a pool of BAD that it concurrently maintains in the phosphorylated, inactive state, rendering survival contingent on sustained SGK activity. Three objectives test this hypothesis.

1. **Establish SGK as a direct BAD kinase.** Map phosphorylation sites using purified components and BAD phosphosite mutants (S112A, S136A, S155A), test endogenous association by reciprocal co-immunoprecipitation, and compare SGK with Akt using selective inhibitors (GSK650394, MK-2206) with P-NDRG1 and P-PRAS40 as selectivity controls.
2. **Define the mechanism of BAD induction.** Quantify endogenous BAD transcript after SGK depletion and glucocorticoid treatment, and distinguish transcriptional activation from altered message or protein stability by promoter reporter, actinomycin D and cycloheximide chase. Test the contribution of CREB, a previously identified SGK substrate.
3. **Determine the survival consequence.** Measure apoptosis by annexin V, caspase-3/7 activity and PARP cleavage after SGK depletion, and test epistasis using BAD knockdown and phospho-mimetic (S112D/S136D) and non-phosphorylatable BAD.

Research design and timeline: Experiments will be conducted in PC-3 (androgen-independent) and LNCaP (androgen-sensitive) prostate carcinoma, H460 lung carcinoma and NMuMG mammary epithelial cells, affording both hormone-responsive and hormone-independent backgrounds. Methods comprise transient transfection, siRNA depletion with two independent sequences and resistant-construct rescue, immunoblotting with phospho-specific antibodies, quantitative PCR against two reference genes, subcellular fractionation and flow cytometry. All findings rest on a minimum of three independent biological replicates; phosphorylation is reported as a phospho:total ratio and total BAD normalised to actin.

Expected outcomes and impact

We anticipate establishing SGK as a bona fide BAD kinase acting alongside Akt, and demonstrating that SGK-driven BAD induction creates a state of conditional dependence in which tumour cells accumulate latent apoptotic potential restrained only by continued SGK signalling. This model predicts that SGK-high tumours are disproportionately sensitive to acute SGK inhibition, and that glucocorticoid co-medication may attenuate the cytotoxic response to chemotherapy — both directly testable and clinically consequential.

Should SGK prove not to phosphorylate BAD in intact cells, the transcriptional arm stands independently and identifies a previously unrecognized route by which hormone-induced kinase signalling sets apoptotic threshold. Either outcome advances understanding of the pathway and is suitable for publication.

The project is composed of discrete, well-controlled experiments suited to undergraduate execution, supporting substantive student training, conference presentation and co-authorship.