Calpain 2 regulates proteolysis of Akt in glioblastoma cell invasion

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INTRODUCTION

Glioblastoma multiforme is a form of primary brain cancer in humans with a 5 year survival rate of less than 4%. Glioblastoma cells are characterized by their highly invasive nature linked to mutations in key signaling proteins such as PTEN, resulting in loss of expression of this lipid phosphatase. When PTEN is not expressed, the phosphorylation and dephosphorylation equilibrium is disturbed resulting in elevated phosphatidylinositol 3,4,5-trisphosphate (PIP3) levels in the cell and constitutive activation of Akt. The Akt pathway, downstream of phosphoinositide 3-kinase production of PIP3, is known to be highly activated and involved in the progression of glioblastoma. Akt is activated by phosphorylation on Thr308 and Ser473 sites in a PIP3-dependent manner. We are examining calpain 2 proteolysis as a novel mechanism for modulating the activity of Akt. Understanding the regulation Akt may lead to new approaches for controlling the activity of a key promoter of cancer progression.

HYPOTHESIS

Calpain 2 directly cleaves Akt in glioma cell invasion

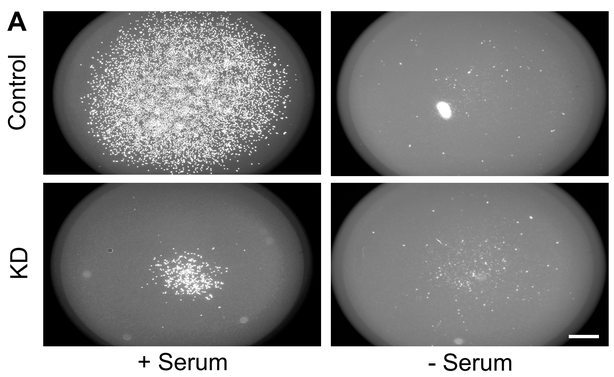

Figure 1. Calpain 2 expression is required for glioblastoma cell invasion. A) Invasion of control and knockdown (KD) cells was measured using Matrigel coated transwells with or without 10% FBS-DMEM (serum) as a chemoattractant in the lower wells. After incubating 3 days, cells on the bottom surface of the transwell membrane were fixed, stained with DAPI, and images captured. **B)** DAPI stained cells were quantified using MetaMorph 6.2. *n*=3 ± SEM. Bar=0.5mm. * Indicates *P*<0.001

RESULTS

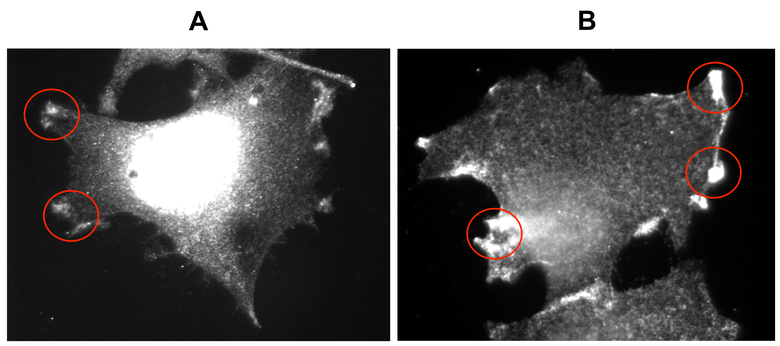

Figure 2. Calpain 2 and Akt localize to membrane ruffles. Glioblastoma cells expressing GFP-calpain 2 **(A)** or stained with anti-Akt pSer473 **(B)** were imaged using fluorescent microscopy. Both Akt and calpain 2 localized to the membrane ruffles representing actin cytoskeletal structures at the leading edge of migrating cells.

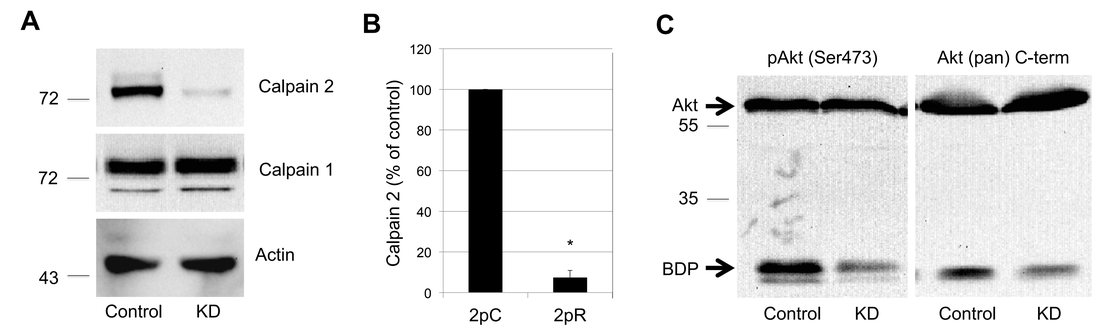

Figure 3. Calpain 2 expression is required for Akt proteolysis. A) Expression of calpain 2, calpain 1, and actin were examined by Western blotting of total cell lysates (50,000 cells). **B)** Bands representing calpain 2 protein were quantified using Molecular Imaging Software v.4.0. *n* = 3 ± SEM. * Indicates *P* = 0.001. **C)** U87MG cell lysates (200,000 cells) were imunoblotted and probed for pSer473-Akt and the C-terminus of Akt. The Akt breakdown product (BDP) was estimated to be 22kDa.

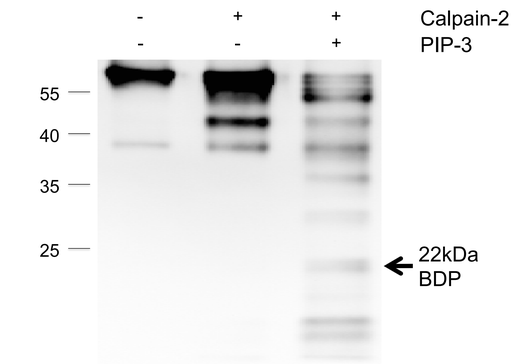

Figure 4. PIP3 binding to Akt enhances susceptibility to calpain 2 proteolysis. Purified recombinant Akt was pre-incubated with PIP3 for 15 min. at 37°C and calpain 2 added for 30 min. Akt protein was identified by Western blotting with anti-Akt C-terminus revealing a 22kDa fragment as observed in **Figure 3C**. Other breakdown products were also observed potentially resulting from *in vitro* proteolysis of purified recombinant Akt with no posttranslational modifications. Results are representative of *n*=3.

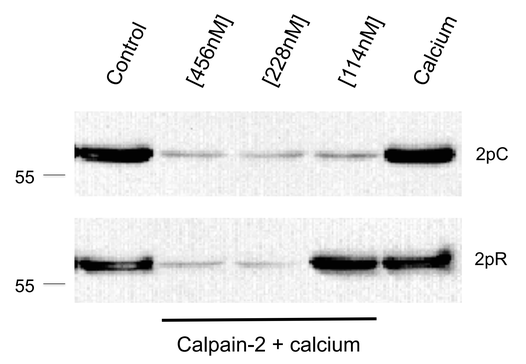

Figure 5. Akt from calpain 2 knockdown cells had decreased susceptibility to calpain 2 proteolysis. 2pC (control) and 2pR (knockdown) cell lysates were treated with three different concentrations of calpain 2 *in vitro*. Reactions began by adding calcium chloride to activate calpain 2 and then incubated at room temperature (25ºC) for 30 min. Akt protein was identified by Western blotting with anti-Akt N-terminus. Results are representative of three independent experiments.

SUMMARY

- Calpain 2 is required for glioblastoma invasion.
- Calpain 2 and Akt both localize to membrane ruffles
- Calpain 2 proteolyzes Akt
- PIP3 binding to Akt enhances proteolysis by calpain 2.

WORKING MODEL

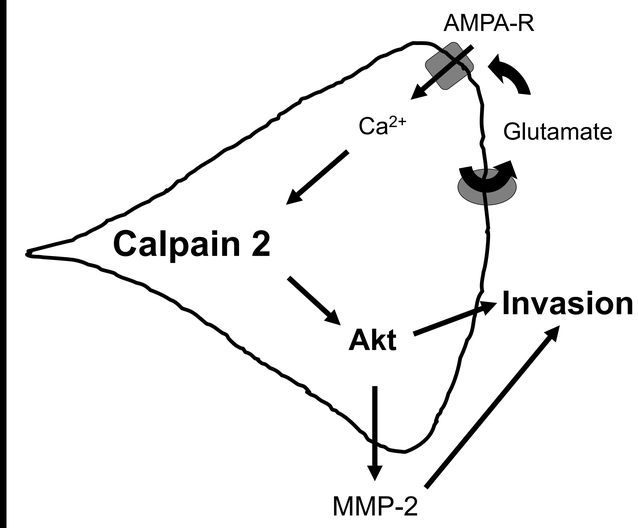

Figure 6. Working model for the role of Akt in glioblastoma cell invasion. Diagram illustrates stimulation of calpain 2 by calcium influxes. We propose that calpain 2 regulates invasion of cells through Akt and extracellular MMP-2.

FUTURE DIRECTIONS

We propose that calpain 2 regulates Akt through phosphorylation and direct cleavage. If this is true, the next step will be to map the exact site of calpain 2 proteolysis. We are also determining which isoform of Akt is being regulated by calpain 2. It has been reported that both calpain 2 and Akt regulate the expression of matrix metalloproteinase-2 required for invasion, although the mechanisms have not been elucidated. We hypothesize that the Akt is cleaved by calpain 2 at the leading edge of invading glioblastoma cells and the breakdown product is signaling to the nucleus to regulate MMP-2 gene expression.

ACKNOWLEDGEMENTS

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