ARF6 Inhibitors for Cancer

February, 2018

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ARF6 Supports Tumor Growth

- ADP ribosylation factor 6 (ARF6) is a small GTPase in the RAS superfamily
- Due to its central role in protein trafficking within cells, ARF6 regulates diverse cellular functions
  - ARF6 enables tumor growth in various cancer types by mediating excessive oncogene signaling, such as a high level of GNAQ activity in uveal melanoma
- ARF6 subserves tumor growth in uveal melanoma, malignant sarcoma, cutaneous melanoma, breast cancer, and ARF6 is implicated in pancreatic cancer
- Pharmacologic inhibition at therapeutic levels is well-tolerated

ARF6 Inhibition in Uveal Melanoma

Uveal Melanoma (UM) Is a Deadly Cancer

- Most common primary ocular malignancy
  - Incidence: 6 per million; 3-5% of melanomas
  - Orphan disease
- Half of patients develop metastases (usually liver)
- No effective treatment for metastatic UM
- Genetic landscape of UM only recently characterized
  - Mutations in $G\alpha_q$ genes GNAQ or GNA11 occur in ~90% of UM cases
  - $G\alpha_q$ proteins transmit growth signals from the cell surface to the nucleus
  - Oncogenic mutations in GNAQ/GNA11 are present in 5.6% of all tumors
- ARF6 controls GNAQ trafficking between the plasma membrane and cytoplasm thereby regulating oncogenic GNAQ signaling
- ARF6 is an actionable target for uveal melanoma

http://www.kimmelcancercenter.org/

ARF6 Knockdown Inhibits Proliferation and Anchorage-Independent Growth of UM Cells

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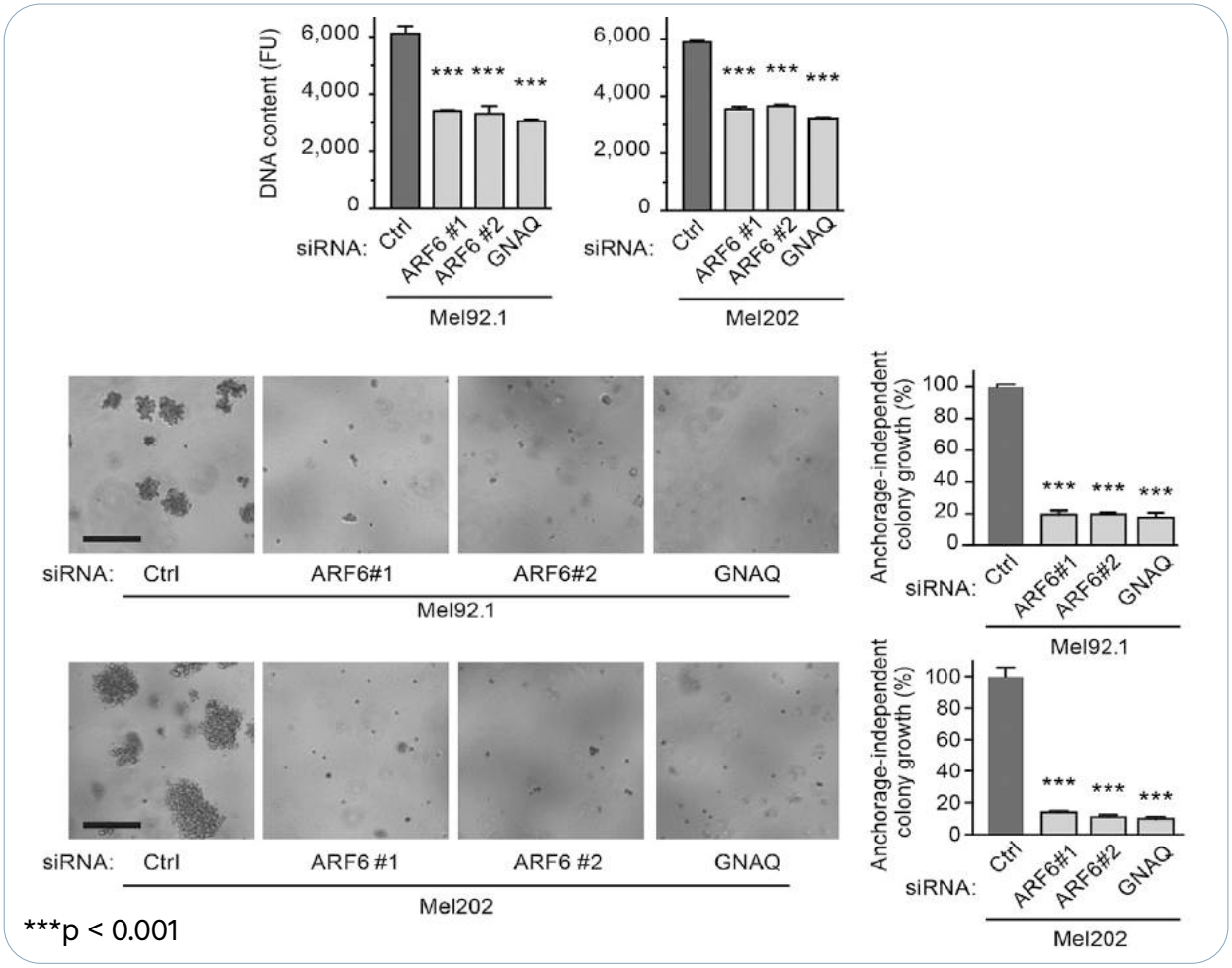

Yoo et al., Cancer Cell 29:889 (2016)

NAV-2729 Inhibits Anchorage-Independent Growth of Uveal Melanoma Cells

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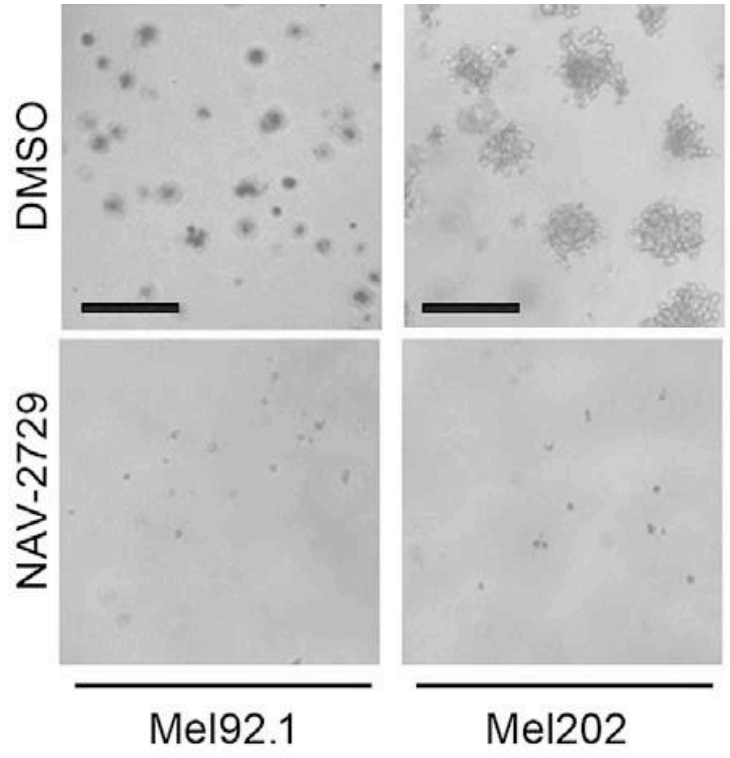

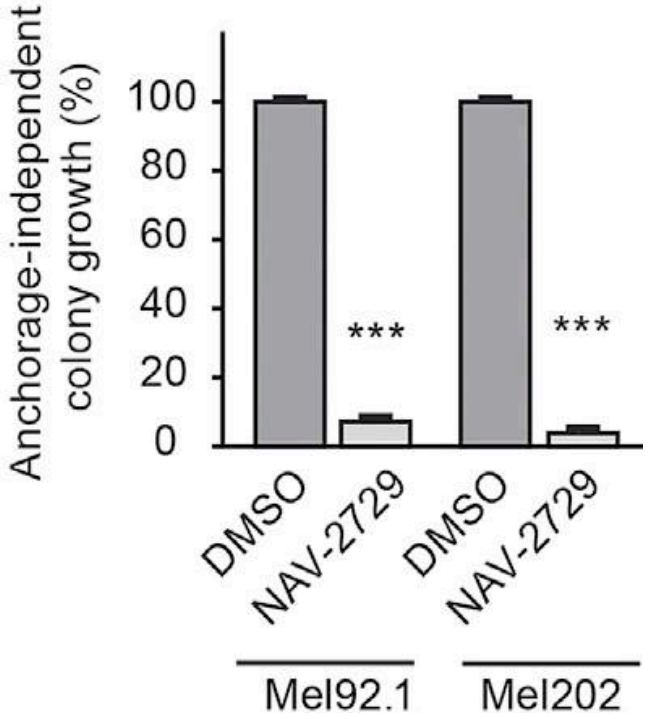

***p < 0.001

Yoo et al., Cancer Cell 29:889 (2016)

ARF6 Knockdown or Pharmacologic Inhibition Significantly Inhibits UM Cell Invasion

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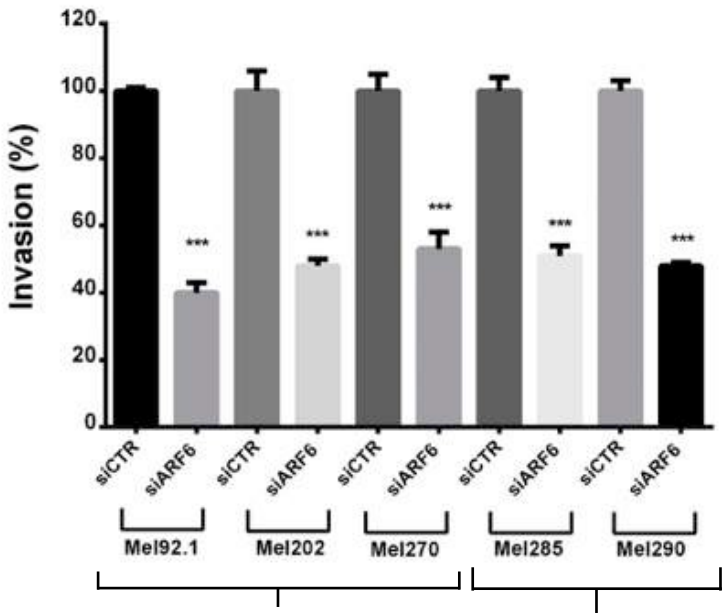

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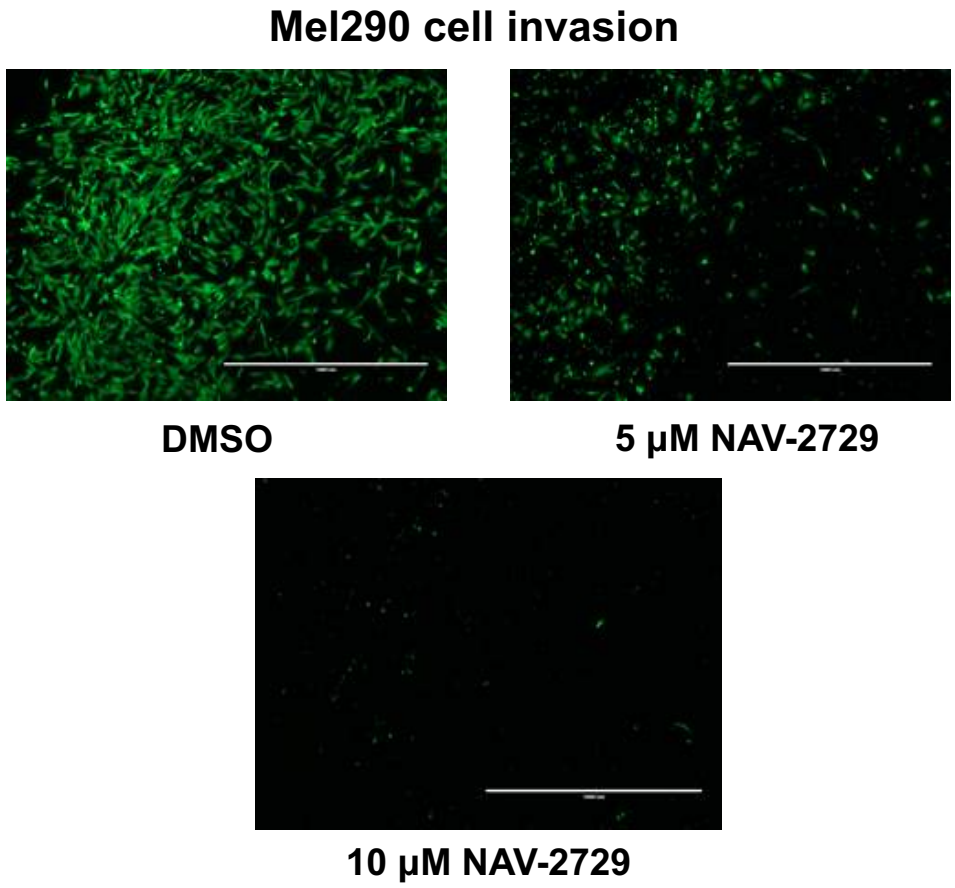

ARF6 Silencing or Pharmacologic Inhibition with NAV-2729 Blocks Tumor Formation and Growth

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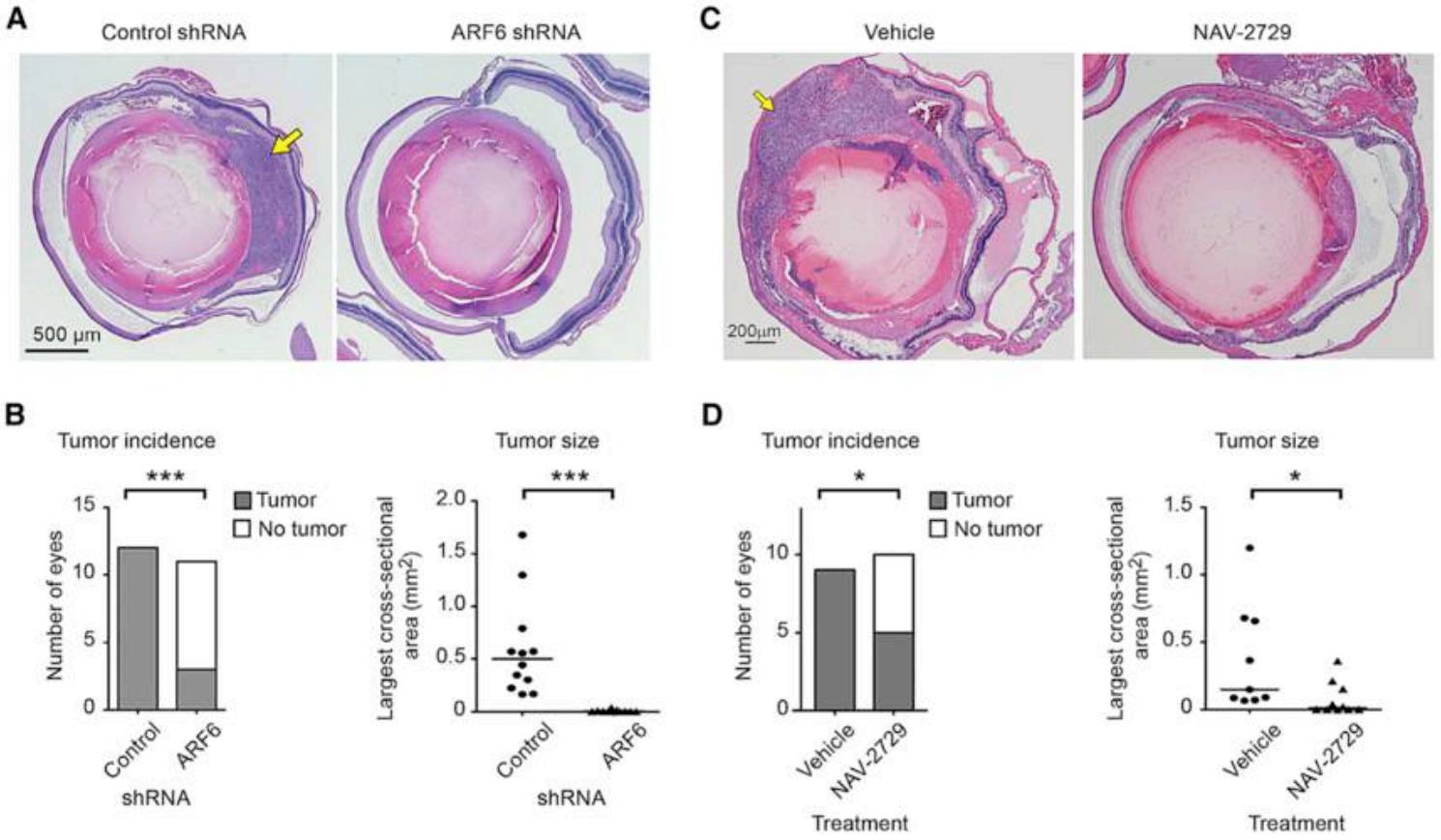
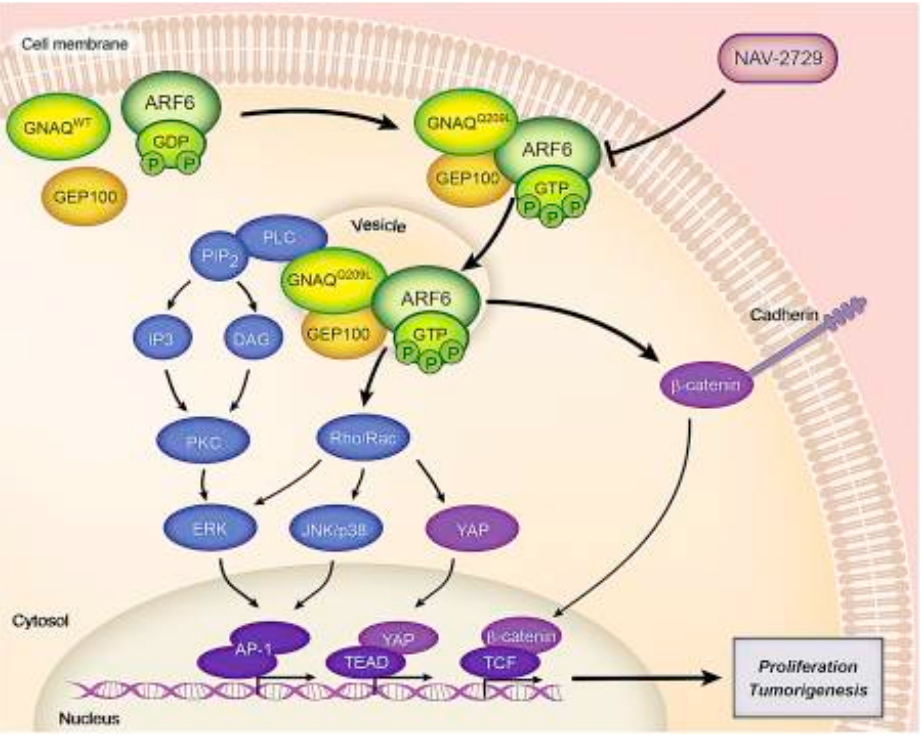


Yoo et al., Cancer Cell 29:889 (2016)

Model of ARF6 Function in Uveal Melanoma

- ARF6 coordinates and maximizes mutant GNAQ signaling by trafficking GNAQ to cytoplasmic vesicles
- ARF6 also regulates β-catenin trafficking in uveal melanoma cells
- Pharmacologic inhibition of mutant GNAQ (Q209L) has not been achieved previously



- Targeting nodes such as ARF6 provides an approach for treating cancers that are not currently amenable to pharmacologic intervention

Yoo et al., Cancer Cell 29:889 (2016)

ARF6 Inhibition in Malignant Sarcoma

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Malignant Peripheral Nerve Sheath Tumors

- Cancer originating from peripheral nerves or connective tissue surrounding nerves
  - Incidence: 10 per million; orphan disease
  - 5-year disease-free survival rate - 49%
  - Also known as neurofibrosarcoma or neurosarcoma
- Aggressive tumor with poor prognosis; 20-25% of patients develop metastases (primarily to lung)
- Mutations in NF1 occur in ~50% of cases and is a poor prognostic factor
  - NF1 encodes neurofibromin, a tumor suppressor gene that regulates the activity of RAS, a common oncogene
- ARF6 controls RAS cellular trafficking in NF1-deficient malignant sarcoma
- ARF6 is an actionable target for malignant sarcoma

ARF6 Activation is Necessary for Cell Proliferation in NF1-Deficient Malignant Sarcoma

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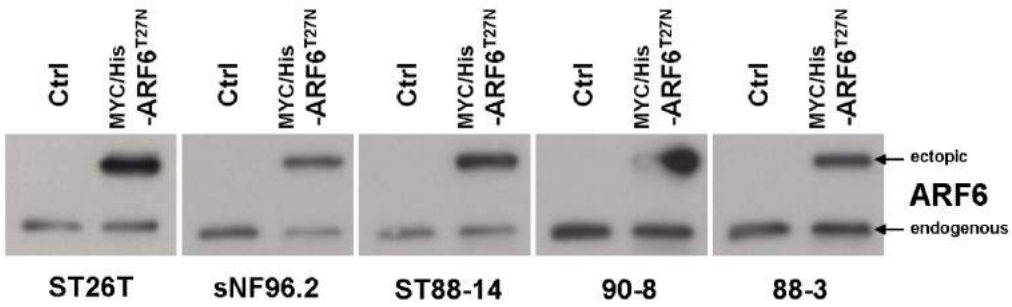
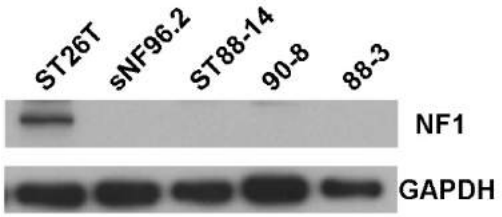
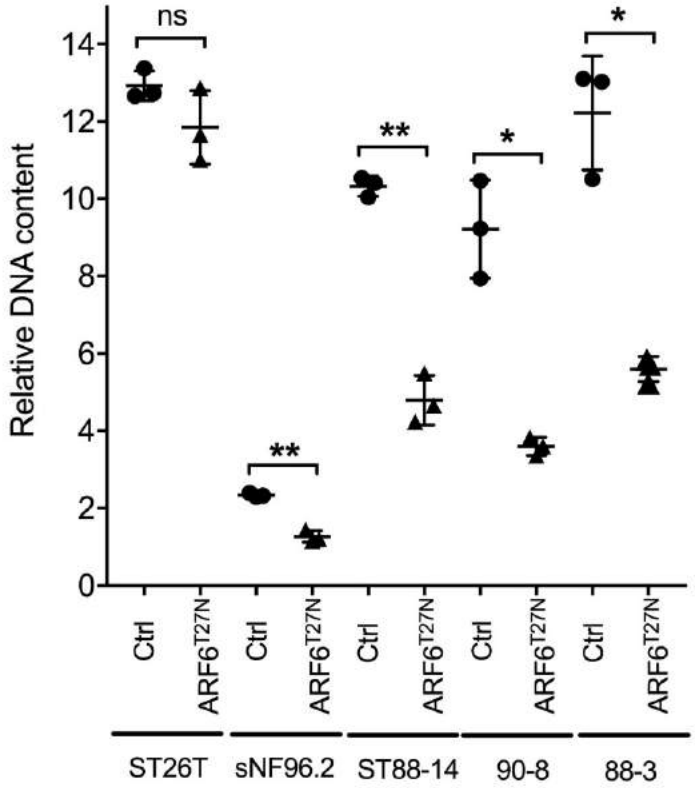
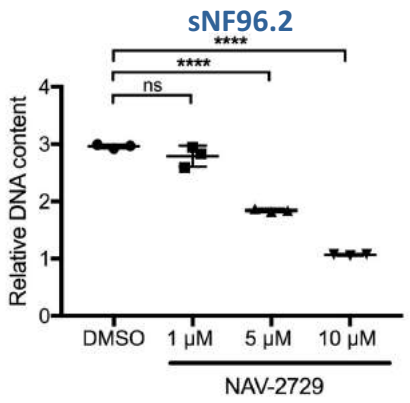


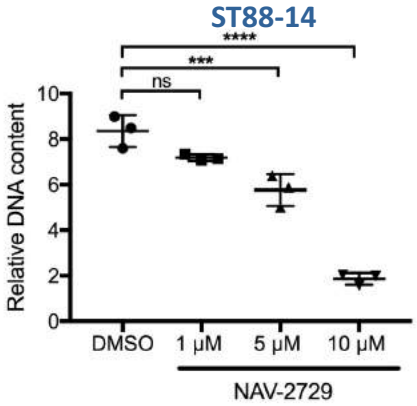


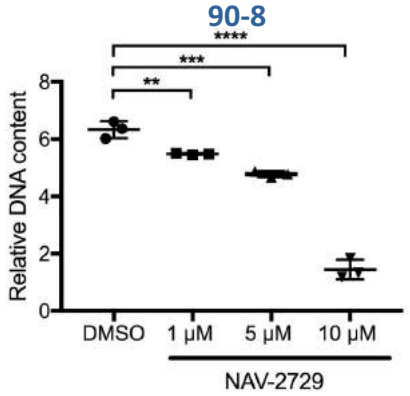
ARF6^{T27N} is a dominant-negative ARF6

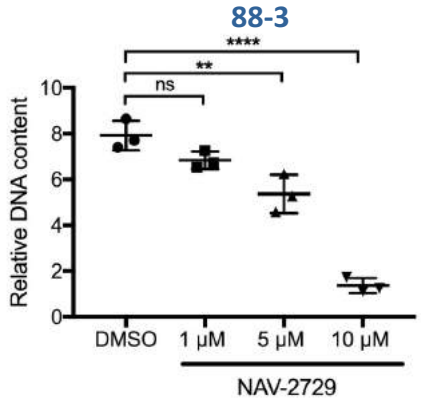


NAV-2729 Inhibits Malignant Sarcoma Cell Proliferation









ARF6 Knockdown Reduces Malignant Sarcoma Tumorigenesis in an Orthotopic Xenograft Model

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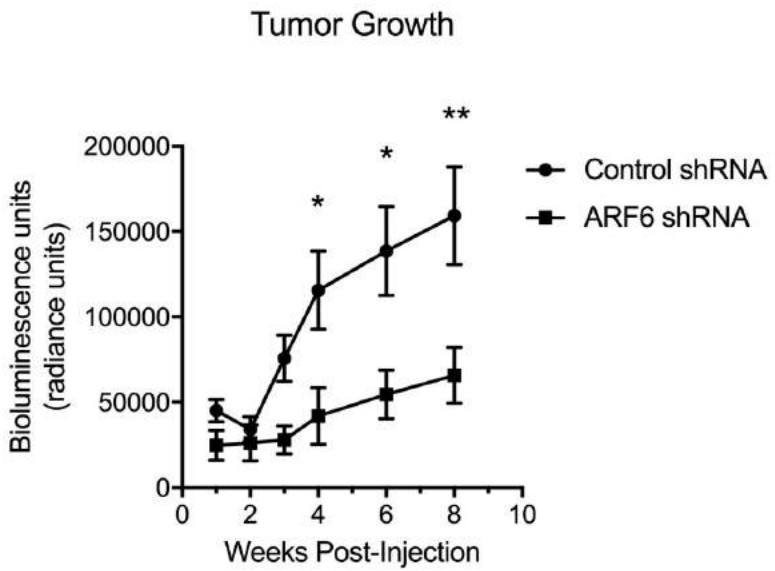

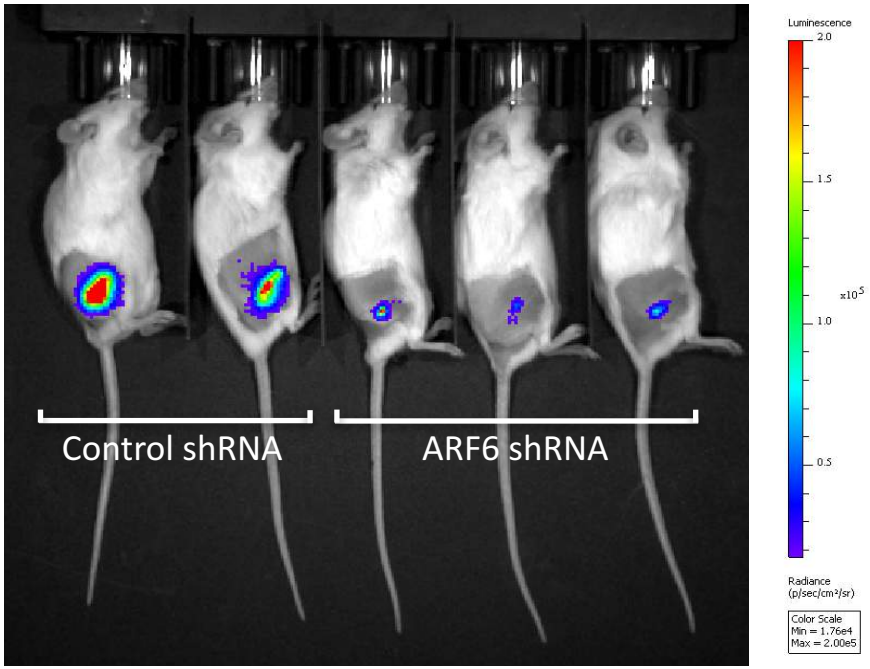

- Luciferase-tagged (bioluminescent) human sarcoma cells (sNF96.2) are orthotopically xenografted into the sciatic nerve of immunodeficient mice
- Graft (tumor) burden is determined by quantitation of luminescence in anesthetized mice

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ARF6 Inhibition in Cutaneous Melanoma

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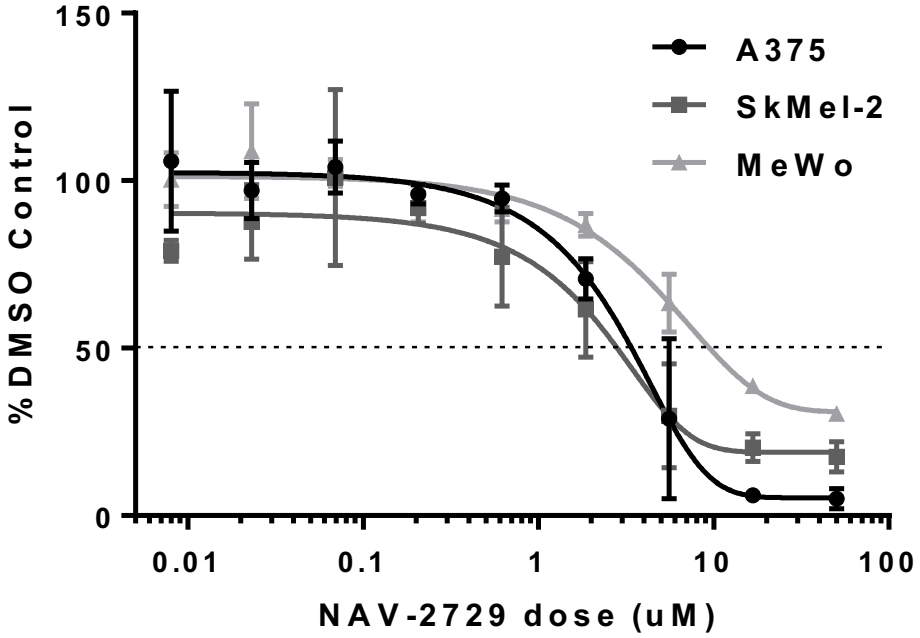
Cutaneous Melanoma

- Cutaneous melanoma is subdivided into subtypes based on the presence of distinct oncogenic drivers: BRAF, RAS, NF1 (loss), or “triple wild-type” (RTK-enriched)
- Mice genetically engineered to harbor melanoma mutations in their melanocytes develop cutaneous melanoma and metastatic disease
- Activated ARF6 supports tumor growth in these melanoma mice, and also gives rise to larger and more numerous metastases

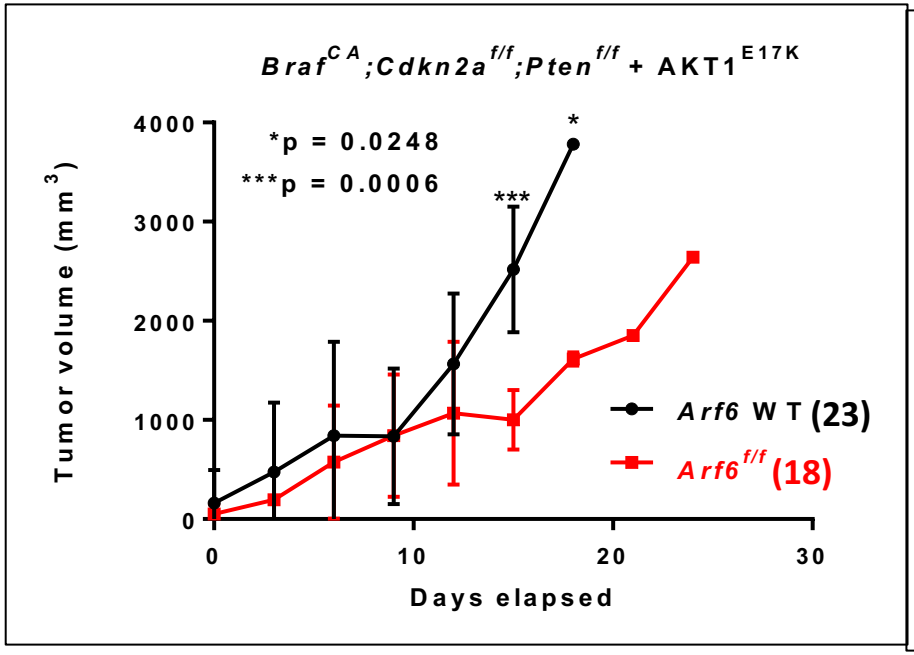
NAV-2729 Inhibits Melanoma Cell Proliferation

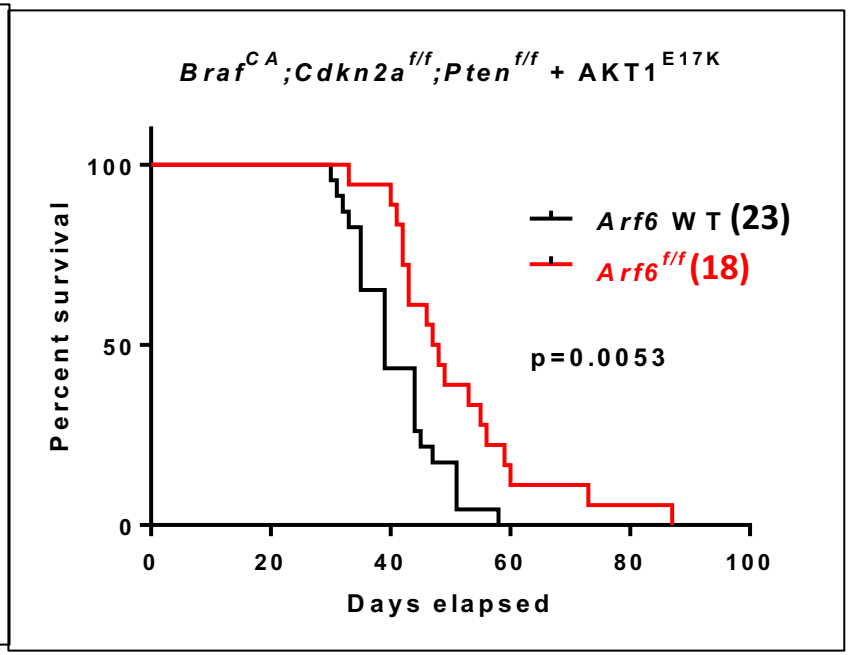
- ARF6 inhibition with NAV-2729 reduces proliferation of human melanoma cell lines with BRAF (A375), NRAS (Sk-Mel-2) and NF1 (MeWo) driver mutations



ARF6 Deletion Inhibits Melanoma Tumor Growth

- ARF6 removal reduces tumor growth (left panel) and increases survival (right panel) in mice with aggressive $BRAF^{V600E}$/$PTEN^{Null}$/$AKT1^{E17K}$-driven tumors





Mice were generated using the RCAS/TVA system, with virus receptor (TVA) under control of the melanocyte-specific promoter dopachrome tautomerase (DCT). Newborn *Dct::TVA;*$Braf^{CA}$*;*$Cdkn2a^{lox/lox}$*;*$Pten^{lox/lox}$*;*$Arf6^{lox/lox}$ mice were injected subcutaneously with RCAS virus encoding Cre alone or in combination with virus encoding $AKT1^{E17K}$. Significance of tumor volume difference (±ARF6) decreases at day 18 due to large primary tumor burden necessitating animal sacrifice.

ARF6-GTP Is Sufficient To Potentiate Metastasis in *Braf^{CA};Cdkn2a$^{f/f}$*-Induced Melanoma

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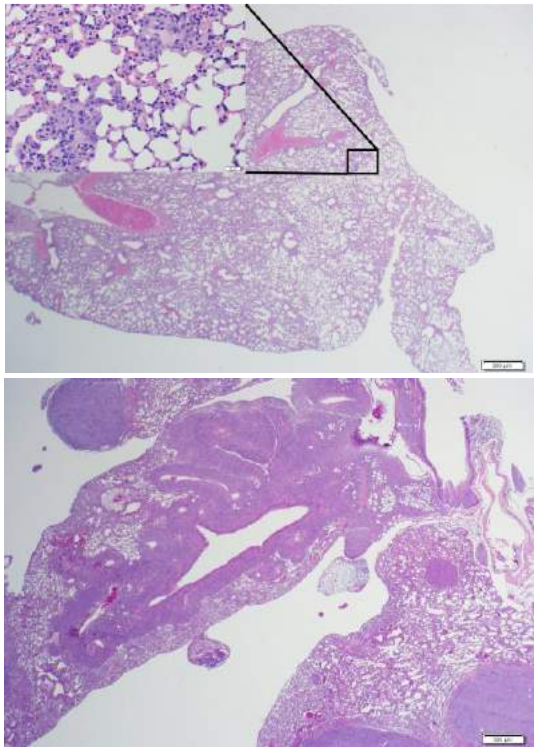
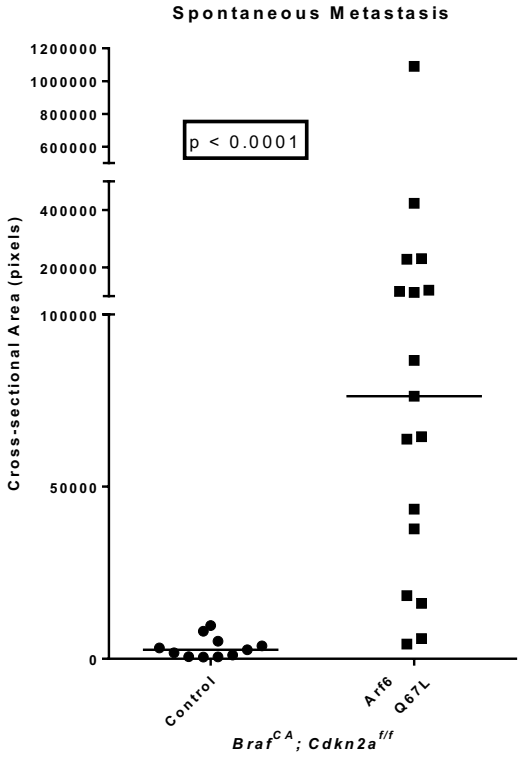
Braf^{CA};Cdkn2a$^{f/f}$

Braf^{CA};Cdkn2a$^{f/f}$ + Arf6^{Q67L}





- Genetically-engineered mice carrying oncogenic BRAF^{V600E} (*Braf^{CA}*) and lacking the cell-cycle control gene (*Cdkn2a$^{f/f}$*) develop metastatic melanoma
- The presence of activated ARF6 (*Arf6^{Q67L}*) increases metastatic burden (larger metastases)

ARF6-GTP Is Sufficient to Facilitate Metastatic Colonization of Circulating Tumor Cells

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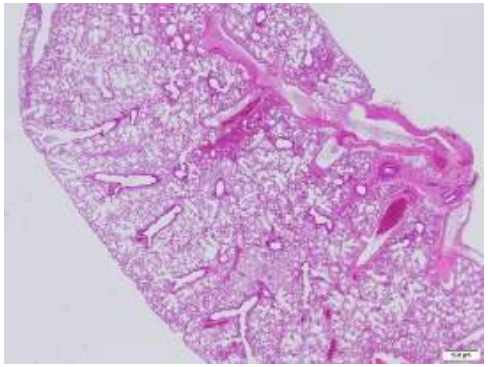
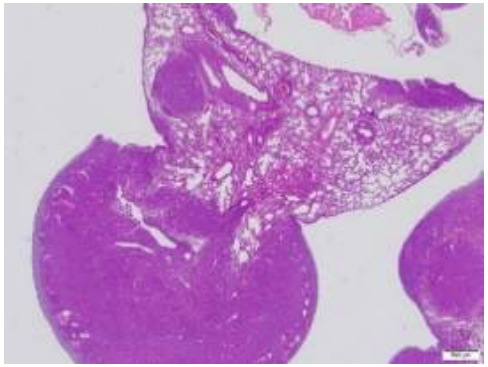
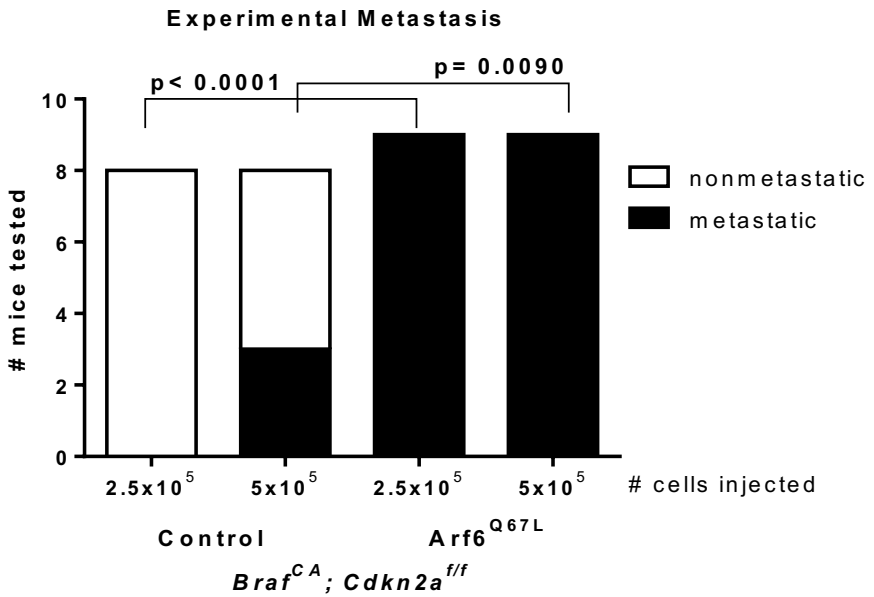
Braf^{CA};Cdkn2a$^{f/f}$



Braf^{CA};Cdkn2a$^{f/f}$ + Arf6^{Q67L}





- Melanoma cells derived from *Braf^{CA};Cdkn2a$^{f/f}$* (control) and *Braf^{CA};Cdkn2a$^{f/f}$;Arf6^{Q67L}* engineered mice were injected into the tail vein of naïve NSG mice.
- Melanoma cells carrying activated ARF6 (*Arf6^{Q67L}*) give rise to more metastases

Pharmacologic Inhibition of ARF6 Reduces Spontaneous Melanoma Metastasis in LOX Xenograft Model

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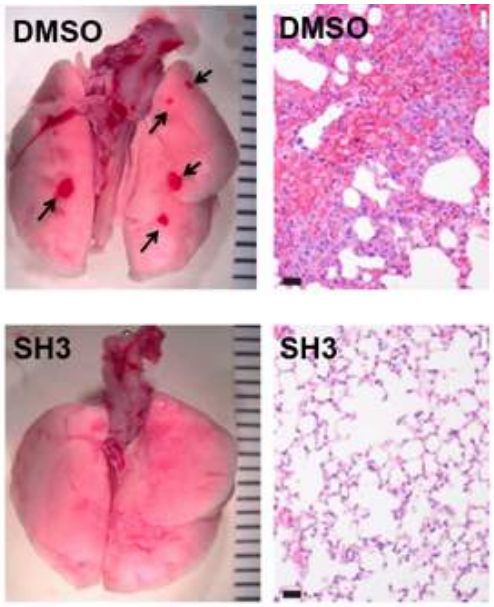
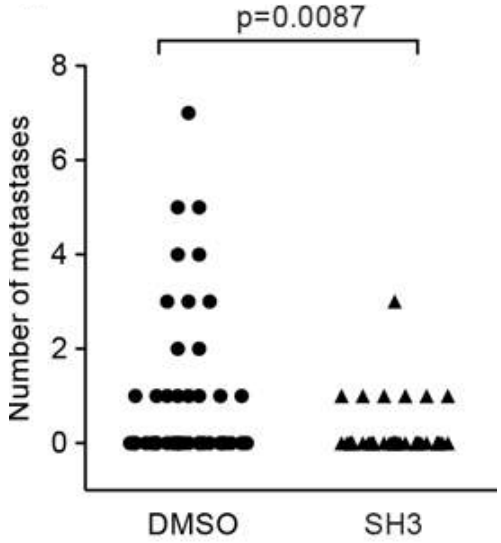
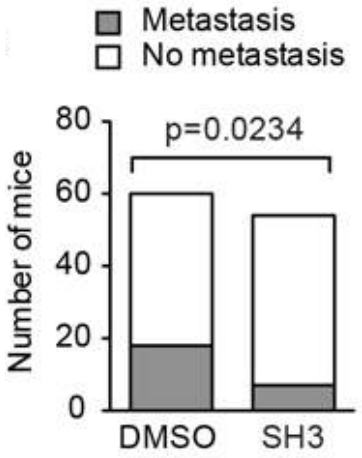
- Subcutaneous LOX xenograft tumors produce spontaneous pulmonary metastases
- ARF6 inhibition was achieved using SecinH3 (SH3), an indirect inhibitor of ARF6







- Blocking ARF6 reduces both the number of metastatic foci and the number of mice with metastasis

Grossmann et al., Science Signaling 6:ra14 (2013)

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ARF6 Inhibition in Breast Cancer

ARF6 and Breast Cancer

- ARF6 protein is overexpressed in malignant breast cancer cells[1]
  - Central role in breast cancer invasion and metastasis
  - ARF6 pathway also disrupts E-cadherin function (cell adhesion)
- Dramatically upregulated in triple-negative breast cancer (TNBC)[2]
  - TNBC is an aggressive disease with a poor prognosis
  - No available molecularly targeted therapy
- Overexpression of GEP100 and AMAP1 (ARF6 pathway) correlates with rapid local recurrence after breast conservative therapy[3]
  - ARF6 is a possible target to block local recurrence

[1]Sabe et al. Cell Adh Migr 2:71; [2]Eades et al. Mol Cancer Res 13:330; [3]Kinoshita et al. PLoS One 8:e76791

ARF6 Inhibition Decreases Triple Negative Breast Cancer Cell Invasion and Proliferation

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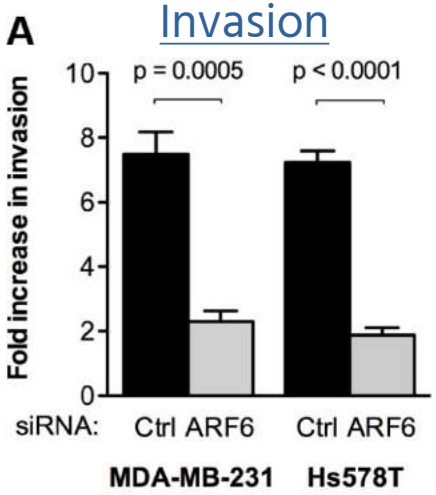
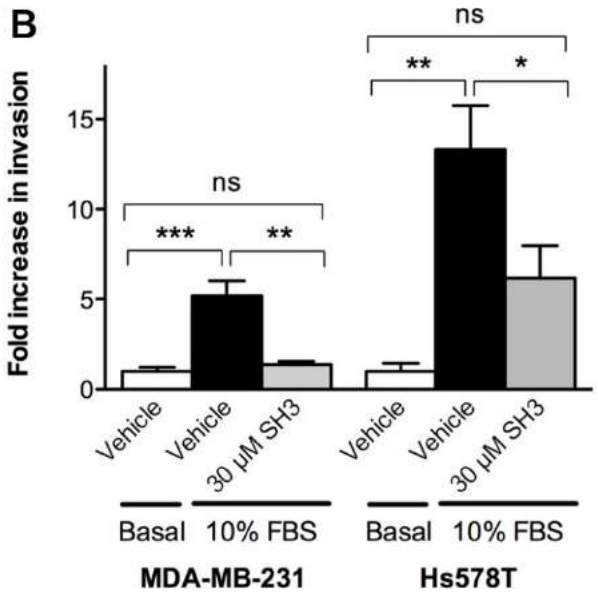
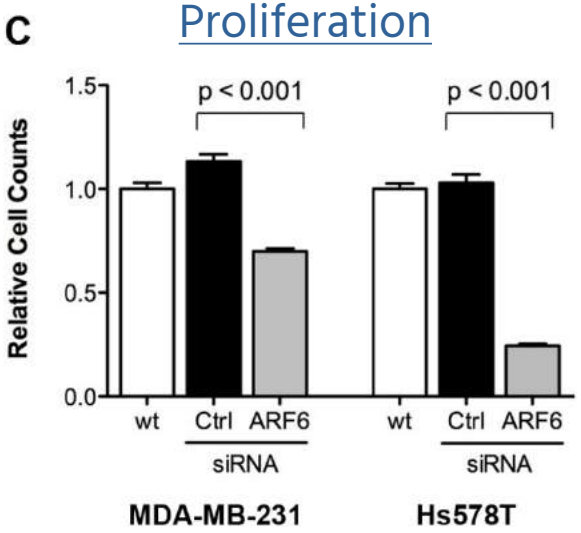
Effect of ARF6 Knockdown on Cell Invasion



Effect of SecinH3 on Cell Invasion



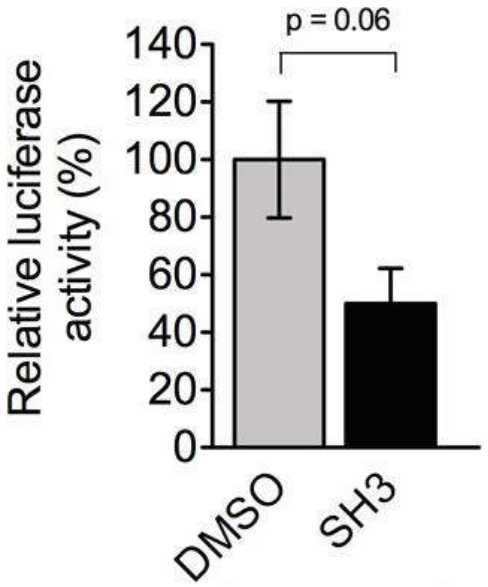
Effect of ARF6 Knockdown on Cell Proliferation



(A) Loss of ARF6 by siRNA decreases breast cancer cell line invasion of Matrigel. Lower compartments contain 5% FBS (except for no FBS controls).
(B) SecinH3 (SH3) inhibits breast cancer cell line invasion of Matrigel. Upper and lower compartments contain vehicle or secinH3; lower compartments contain 10% FBS (except for no FBS controls). SecinH3 is an indirect inhibitor of ARF6.
(C) Loss of ARF6 by siRNA decreases breast cancer cell line proliferation.
MDA-MB-231 and Hs578T are basal-type triple-negative breast cancer cell lines.

ARF6 Inhibition Decreases β-Catenin Signaling

- β-catenin has dual roles as a transcription activator in the nucleus (tumor activation) and an adhesion factor at plasma membrane adherens junctions (tumor suppression).
- ARF6 controls the balance of membrane and nuclear pools of β-catenin: Activated ARF6 mediates membrane release and shuttling of β-catenin to the nucleus.[4]
- In breast cancer, nuclear β-catenin promotes tumorigenic behavior of TNBC cells by controlling migration, stemness, and anchorage-independent growth.[5]



SecinH3 inhibits β-catenin-mediated transcription in MDA-MB-231 cells.
β-Catenin activity was measured using the TOPflash-based 7TFP luciferase reporter containing 7 tandem β-catenin (TCF/LEF) binding sites. SecinH3 (SH3) is an indirect inhibitor of ARF6. DMSO is the vehicle control.

[4]Grossmann et al. Sci Signaling 6:ra14; [5]Xu et al. PLoS One 10:e0117097

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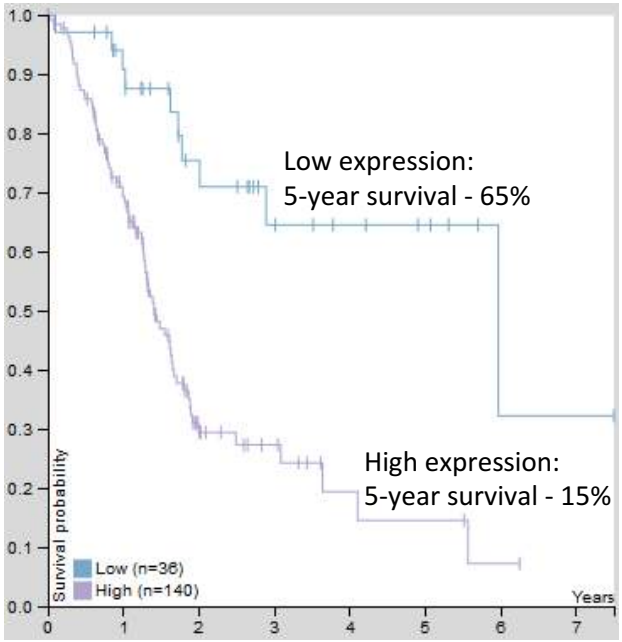
ARF6 Role in Pancreatic Cancer

Pancreatic Ductal Adenocarcinoma (PDAC)

- ARF6 is a central component of the inhibitory SLIT/ROBO embryonic axonal guidance pathway
- Decreased SLIT-ROBO signaling has been implicated in lung, breast, kidney, and cervical cancer[6]
- Recurrent mutations and copy number variation of axon guidance pathway genes in patients with early-stage sporadic PDAC have been identified, suggesting that aberrant SLIT-ROBO signaling is potentially a common feature (5-20%) of PDAC[7]
- Low ARF6 mRNA expression is associated with improved patient survival[8]
  - Patients divided based on expression level into low- or high-expression groups
  - Kaplan-Meier plot summarizes results from analysis of correlation between mRNA expression level and patient survival

Survival Curve: ARF6 Expression



[6]Mehlen et al., Nature Reviews Cancer 11:188 (2011); [7]Biankin et al., Nature 491:399 (2012); [8]Uhlen et al., Science 357:eaan2507 (2017)

Conclusions

- ARF6's role in the intracellular trafficking of multiple growth signaling proteins accounts for its involvement in distinct cancers
- Current evidence implicates ARF6 function in uveal melanoma, malignant sarcoma, cutaneous melanoma, breast cancer, and pancreatic cancer
- A6's ARF6 inhibitors have been shown to be effective in animal models of uveal melanoma, malignant sarcoma, and cutaneous melanoma
- A6 is currently determining the therapeutic utility of ARF6 inhibitors in treating a broad range of cancers

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