Targeted disruption of transcriptional effector GLI2 attenuates breast tumor growth and metastasis

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Abstract

We reported that transcriptional regulation by the major Hedgehog (Hh) pathway effector GLI2 in mammary gland stromal cells coordinates a hormone-responsive niche signaling program that directs epithelial stem cell activity during puberty [Zhao, et al., Science. 2017 Apr 21;356(6335)]. Hh signaling is a key growth pathway in human carcinogenesis. The first targeted therapies aimed at G-protein coupled receptor Smoothened (SMO) resulted in rapid acquired resistance, underscoring the need for developing new HH pathway-targeting therapies downstream of SMO. In breast cancer, we and others reported that Gli-dependent transcription may involve a non-receptor-based mechanism of GLI2 activation involving cross-talk from other signaling pathways such as TGFß, PI3K, Wnt, or NF-κB. Accordingly, we investigated the role of the Hh pathway and its transcriptional effectors using the *MMTV-PyMT* breast cancer mouse model. Development of mammary tumors arising in these mice proceeds through neoplastic lesions ranging from carcinoma in situ (10-12 weeks) to highly invasive ductal carcinoma with high incidence of pulmonary metastasis at 16-18 weeks. Epithelial ablation of *Gli2* dramatically reduced tumor progression. Cre recombinase expressed under control of the *CK14* promoter to genetically ablate *Gli2* in basal cells of the mammary gland thus dramatically attenuated metastasis despite continued (albeit reduced) formation of primary tumors. In human breast cancer, we applied Bayesian methods to gene expression data to identify metastatic breast cancers with Hh pathway activation. Kaplan-Meier analysis demonstrated significantly worse progression-free survival in patients with Hh pathway activity (Log-rank p = 0.0013). Next, we analyzed 1294 breast cancer samples, stratified according to Hh activity, using the Hh probability as a continuous score ranging between 0 and 1. Univariate Cox regression analysis supports the hypothesis that Hh activity in breast cancer is a risk factor for relapse after surgery, HR = 2.45 (95% CI: 1.67 – 3.61, p = 2.72e-6). There is also a significant difference in survival between Hh-actives and Hh-inactives (Log-rank p = 3.91e-4), suggesting a pathogenic role of Gli activation in breas cancer progression and metastasis. Arsenic trioxide (ATO) inhibits Hh pathway activity by de-stabilizing the Gli transcriptional effectors of HH signaling, likely due to arsenic displacement of zinc within the DNA-binding zinc fingers of Gli proteins. ATO treatment of BC cell lines resulted in dose-dependent cell growth inhibition and induction of apoptosis (PARP cleavage and Annexin V expression) at clinically achievable concentrations. In NSG mice with orthotopic transplant of 3e5 SU151 human breast cancer patient-derived xenografts, with strongly positive Gli-active signature by Bayesian analysis, daily IP injection of 10mg/kg ATO resulted in marked tumor growth inhibition *in vivo* (2.75-fold smaller tumor diameter vs. PBS vehicle controls, p = 0.006; 55d vs. 17d to reach 1.5 cm criterion for ATO vs. vehicle). We conclude that the major Hh pathway transcriptional effector GLI2 coordinates a transcriptional program with a central role in breast cancer growth and metastasis in a significant subset of BC, and that systemic treatment with ATO (new oral formulation in commercial development), will reduce metastatic progression and improve clinical outcomes in patients whose tumors harbor a Gli-active transcript signature.

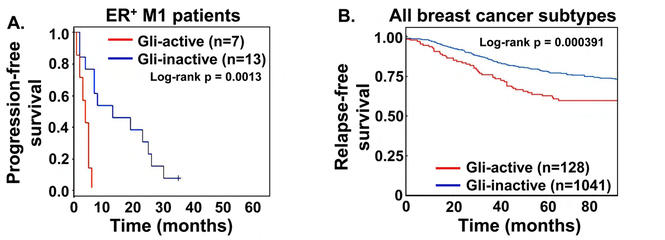

Figure 1: Gli activity predicts poor breast cancer prognosis.
A Bayesian network analysis developed by Philips Research results in a gene expression signature which assesses the likelihood of Hh/Gli pathway activity. A Gli-active gene expression signature is associated with worsened prognosis in patients with metastatic, ER+ breast cancer at diagnosis (ER+ M1) **(A)** or in a cohort containing all breast cancer subtypes **(B)**.

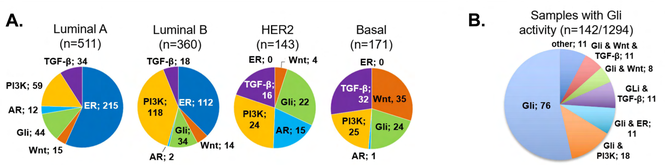

Figure 2: Gli activity is detected in all breast cancer subtypes.
Using publicly available datasets, Bayesian network analysis assesses signaling pathway activity in breast cancer samples. A Gli-active gene expression signature is detectable in some breast cancers of all major molecular subtypes (green region) **(A)**. Of cancers with Hh/Gli pathway activity, half also show activity of additional signaling pathways **(B)**.

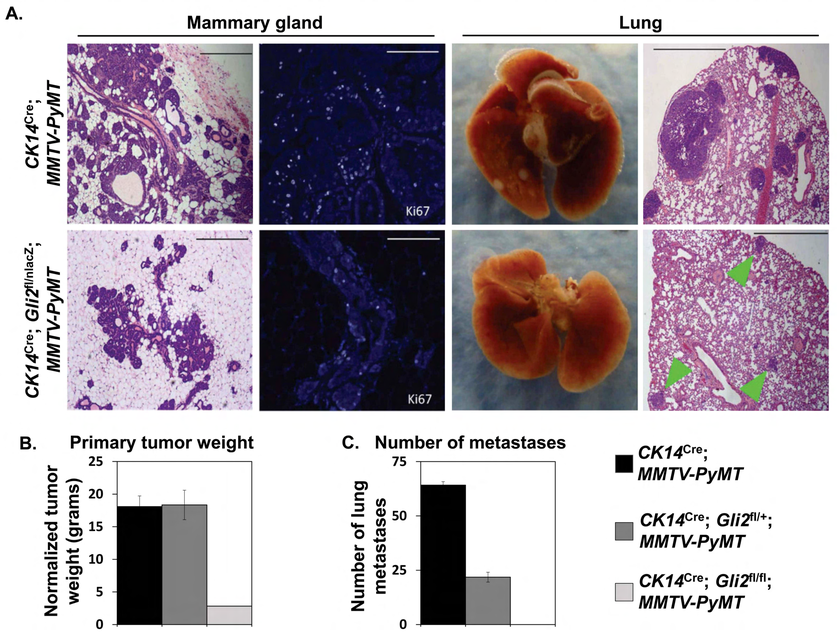

Figure 3: Epithelial *Gli2* promotes mammary tumor progression.
Abolition of Hh/Gli pathway activity in mammary epithelial cells through *CK14*^{Cre}-mediated recombination of *Gli2* in the *MMTV-PyMT* mouse model of breast cancer. Ablation of both *Gli2* alleles reduces primary tumor growth and proliferation (assessed by Ki67 staining) **(A, B)**. Further, metastasis is exquisitely sensitive to *Gli2* gene dosage. Although loss of a single copy of *Gli2* has no impact on primary tumor size **(B)**, metastatic outgrowth is dramatically reduced **(C)**. Loss of both *Gli2* alleles results in nearly complete elimination of lung metastases **(A, C).**

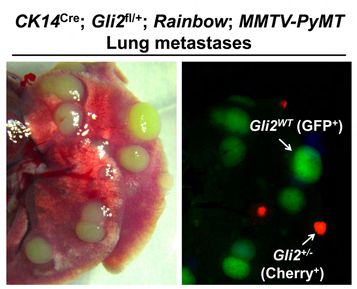

Figure 4: Heterozygous *Gli2* knockout impairs metastasis.
In epithelial *Gli2* heterozygous mice in which recombination is monitored with the Rainbow allele, large metastatic lesions are GFP+, indicating failed recombination and a *Gli2* wild-type genotype. *Gli2* heterozygous metastases, marked by Cherry+, are smaller and less frequent.

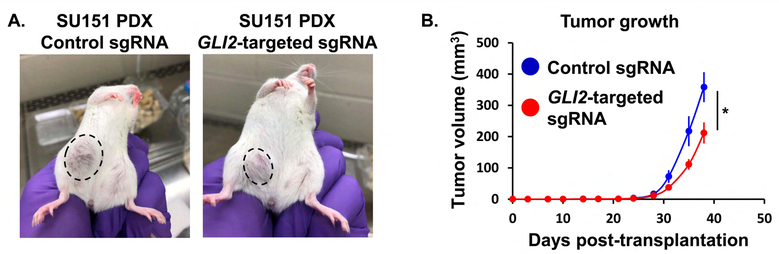

Figure 5: *GLI2* knockout reduces growth of orthotopically transplanted tumors.
Growth of a Gli-active human patient-derived xenograft (PDX) tumor, SU151, is augmented by Hh/Gli pathway activity. CRISPR/Cas9-mediated ablation of the essential Hh pathway transcription factor *GLI2* results in impaired tumor growth **(A, B)**. Error bars represent SEM, p < 0.05.

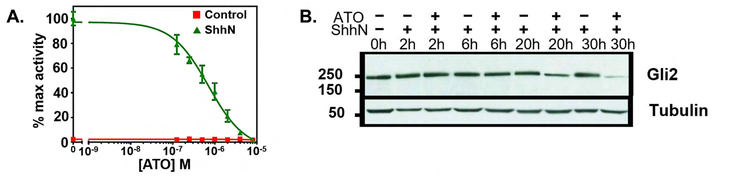

Figure 6: An arsenic-based therapeutic inhibits Gli activity.
Arsenic trioxide (ATO), an FDA-approved anti-leukemia drug, inhibits Hh/Gli pathway activity as monitored using a Gli-dependent reporter **(A)**. Unlike FDA-approved compounds which target SMO, a Hh pathway component upstream of Gli2, ATO directly promotes Gli2 degradation **(B).** [Kim, et al., PNAS. 2010 Jul 12;107(30)]

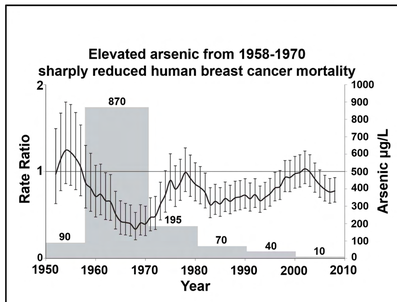

Figure 7: Exposure to high levels of arsenic is associated with reduced breast cancer mortality.
Breast cancer mortality was sharply reduced during the decade in which a population in the Atacama Desert in Chile was exposed to high levels of arsenic through the drinking water. Mortality is shown as the ratio to mortality in a control Chilean population with normal arsenic consumption. [Smith, et al., EBioMedicine. 2014 Nov 1;1(1)]

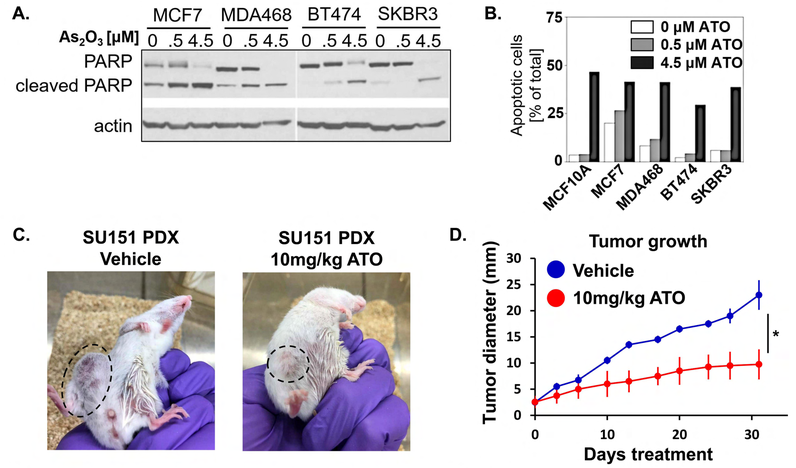

Figure 8: ATO inhibits breast cancer proliferation.
Treatment of breast cancer cell lines with ATO for 72 hours results in apoptosis, as evaluated by PARP cleavage **(A)** or by Annexin V staining **(B)**. Further, SU151 xenograft growth is significantly impaired by daily systemic treatment with ATO **(C, D)**. Error bars represent SEM, p < 0.01. A and B: [Smith, et al., EBioMedicine. 2014 Nov 1;1(1)]

Conclusions

- A Gli-active gene expression signature is associated with a more dire prognosis in breast cancer.
- We find an association between epithelial Hh/Gli pathway activity and metastasis, the primary cause of breast cancer mortality, and are are investigating the mechanistic basis of this association.
- Genetic or pharmacologic Gli inhibition impairs breast cancer growth. Several Hh/Gli pathway antagonists, including ATO, are FDA-approved, well-tolerated anti-cancer therapeutics.
- A clinical trial of Hh/Gli pathway antagonists in breast cancer patients may be warranted. Stratification through our Bayesian network analysis may help identify patients within all breast cancer subtypes who may benefit from these drugs.