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14. ABSTRACT Melanoma is the most aggressive and lethal form of skin cancer. In 2013 over 75,000 Americans were diagnosed with melanoma, and nearly 10,000 died from this disease. It has been known for over a decade that mutations that overactivate the <i>BRAF</i> and <i>NRAS</i> genes promote melanoma formation. At the same time it has also become clear that these mutations are not sufficient for melanoma formation and other genes are involved. Using genomic studies and cross-species comparisons, we identified the BMP factor <i>GDF6</i> as a gene that may cooperate with mutant <i>BRAF</i> to promote melanoma. The aims of this grant are to determine if <i>GDF6</i> does in fact cooperate with mutant <i>BRAF</i> and uncover the mechanisms by which <i>GDF6</i> acts in melanomas and normal melanocytes. Toward these aims, we have used our zebrafish model to demonstrate cooperativity between <i>GDF6</i> and mutant <i>BRAF</i> in accelerating melanoma onset. Furthermore, we have knocked down <i>GDF6</i> in human melanoma cells, finding that loss of <i>GDF6</i> causes cells to cease proliferating. These and other data suggest that <i>GDF6</i> promotes melanoma progression and its withdrawal is detrimental to melanoma cell growth. We are currently investigating whether blocking <i>GDF6</i> function is a viable therapeutic strategy.					
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INTRODUCTION:

Melanoma is the most aggressive skin cancer, and every year it kills nearly 10,000 Americans and roughly 60,000 people worldwide. A greater understanding of the genetic basis for melanoma is essential for designing new ways to diagnose and treat this disease. Nearly a decade ago, it was discovered that mutations that inappropriately activate the *BRAF* gene are present in over half of all human melanomas. Activated *BRAF* mutations are necessary for formation of these melanomas, but numerous studies have shown that they are not sufficient. To find other genes that cooperate with *BRAF* in creating melanomas, we have used genomic studies and cross-species comparisons to identify several candidates. One of these candidates, *GDF6*, is a BMP factor that is recurrently amplified and upregulated in human and zebrafish melanomas. The purpose of this study is to functionally analyze the role of *GDF6* in melanoma progression. In addition, this study aims to use gain and loss of function studies to determine how *GDF6* acts in melanomas and normal melanocytes. A major goal of this research is to determine if *GDF6* can be used as a diagnostic or prognostic marker in melanoma and is a potential therapeutic target.

BODY:

As requested in the Technical Reporting Requirements, this section describes research progress in reference to each task outlined in the Statement of Work. Below, I restate each task and briefly describe its components. With each task an update on progress made is included.

Task 1: Perform gain and loss of function studies in zebrafish embryos and mammalian cultured cells to determine if GDF6 antagonizes melanocyte development.

In this task, studies in zebrafish and mammalian cultured cells were proposed to determine the effects of *gdf6b* overexpression and *gdf6b* loss on melanocyte development. Zebrafish expressing *gdf6b* in melanocyte progenitors fail to develop melanocytes, suggesting that *gdf6b* inhibits terminal differentiation of melanocytes. We have created a zebrafish strain with a targeted mutation in *gdf6b*. *gdf6b* mutant animals have excess melanocytes, consistent with a role for *gdf6b* in inhibiting melanocyte development. These animals are being further characterized to determine the stage of melanocyte development during which *gdf6b* acts. We have also performed knockdown and overexpression experiments in human cultured melanoma cells. As described below, alteration of *GDF6* levels has profound effects on cell viability and tumorigenic potential.

Task 2: Use established screening procedures in zebrafish to determine if GDF6 overexpression accelerates melanoma onset or exacerbates other properties of melanomas. In addition, use human melanoma cells to determine if GDF6 knockdown in GDF6-positive cells or overexpression in GDF6-minus cells affects tumorigenicity.

To address this task, a zebrafish screening scheme, termed the 'MiniCoopR' assay^{1,2}, was used to determine if *gdf6b* has an effect on melanoma progression. In this assay, melanocyte-deficient animals are injected with DNA that can both rescue melanocytes and overexpress a gene of interest. Zebrafish with rescued melanocytes are monitored weekly for tumors to determine if the gene of interest affects tumor onset as compared to a control gene. When *gdf6b* was overexpressed using MiniCoopR, melanomas arose more quickly as compared to *EGFP* controls (Fig. 1A). *GDF6* was also expressed in cultured human A375 melanoma cells. *GDF6* overexpressing cells were xenotransplanted into nude mice and tumor progression monitored as compared to control A375 cells. *GDF6* overexpression caused tumors to grow much more quickly than controls (Fig. 1B,C). A375 and other human melanoma cell lines express endogenous *GDF6*, so we determined the effects of *GDF6* knockdown in these cell lines. When *GDF6* was knocked down using multiple, independent shRNAs A375 and other melanoma cells underwent programmed cell death (Fig. 2). When knockdown cells were xenotransplanted prior to death, melanoma progression was markedly decreased (Fig. 3). Taken together, these results indicate that *GDF6* is an oncogene and the cell death resulting from its knockdown makes it an excellent target for anti-melanoma therapy.

Knockdown and overexpression cells are being used to determine how *GDF6* acts. The *GDF6* protein is initially made as a proprotein, which is cleaved in cells to generate mature, secreted *GDF6*³. To determine if mature *GDF6* acts as a pro-survival factor, we added recombinant, mature *GDF6* to media of *GDF6* knockdown cells. Recombinant *GDF6* rescued the effects of *GDF6* knockdown (Fig. 4), indicating that *GDF6* can act as a secreted protein to promote melanoma cell survival. These data suggest that targeting soluble, extracellular *GDF6* is a therapeutic strategy for melanoma and possibly other types of tumors. Currently we are characterizing the transcriptomes of *GDF6* to further address the effects of *GDF6* knockdown and overexpression. Analyses thus far are consistent with a role for *GDF6* in promoting cell survival.

Task 3: Use BMP pathway reporters to determine the dynamics of BMP activity in normal melanocytes and melanoma cells. Examine GDF6 expression and mutation status in human melanomas, benign melanocytic lesions and normal melanocytes to determine if modulation of GDF6 activity is consistent with a role in melanoma formation.

A major goal of this task is to assess the effects of *GDF6* on BMP signaling activity. In zebrafish we initially proposed to use a fluorescent reporter to monitor transcriptional output of BMP activity – however, technical difficulties have made this approach untenable. Instead, we have used antibodies that recognized phosphorylated SMAD1/5/8 to measure BMP signaling activity. In zebrafish, melanomas have high levels of *GDF6* protein as well as robust phospho-SMAD expression (Fig. 5). In cultured melanoma cells we similarly detect *GDF6* and phospho-SMAD1/5/8 expression. When *GDF6* is knocked down, phospho-SMAD1/5/8 levels go down (Fig 6), consistent with the notion that *GDF6* signals through SMAD1/5/8 and the BMP signaling pathway.

Additional experiments were performed to determine if *GDF6* acts via the BMP signaling pathway. Knockdown of *SMAD1* resulted in the same cell death phenotype as *GDF6* knockdown, suggesting that both genes act in the same pathway. To directly assess whether *GDF6* acts via the BMP signaling pathway we performed genetic epistasis analyses (Fig. 7). In these epistasis experiments an activated variant of *SMAD1* was used. This variant, *SMAD1DVD*, contains amino acid substitutions in key catalytic residues, resulting in a constitutively active protein⁴. When *GDF6* knockdown was performed in A375 cells expressing *SMAD1DVD*, cell death was suppressed, indicating that *GDF6* acts upstream of or in parallel to *SMAD1*. When such cells were xenotransplanted into immunocompromised mice, they grew much more quickly than *GDF6* knockdown cells, again indicating that *GDF6* acts upstream of *SMAD1*.

Stainings of zebrafish and human tissue samples were used to further investigate the role of *GDF6* and *SMAD1* in melanoma (Fig. 8). In zebrafish melanomas we discovered robust expression of *GDF6* and phospho-*SMAD1*. Similar, robust expression of *GDF6* and phospho-*SMAD1* was observed in human melanoma sections. To determine if there is a correlation between *GDF6* or phospho-*SMAD1* expression and clinical outcome, we have recently stained a tissue microarray of human melanoma tissue cores, each of which has associated clinical data. We are currently analyzing these stainings to determine if increased *GDF6* or phospho-*SMAD1* expression correlates with a poor clinical outcome.

KEY RESEARCH ACCOMPLISHMENTS:

- Overexpression of GDF6 accelerates melanoma onset in zebrafish.
- Overexpression of GDF6 accelerates melanoma onset of A375 cells in xenotransplanted mice.
- Knockdown of *GDF6* causes programmed cell death.
- Recombinant GDF6 protein rescues effects of *GDF6* knockdown.
- Knockdown of *SMAD1* causes programmed cell death.
- Expression of an activated *SMAD1* variant, *SMAD1DVD*, suppresses the effects of *GDF6* knockdown.
- GDF6 and phospho-SMAD1 are robustly expressed in zebrafish melanomas.
- GDF6 and phospho-SMAD1 are robustly expressed in human melanomas.
- Zebrafish with mutant GDF6 have supernumerary melanocytes.

REPORTABLE OUTCOMES:

Presentations during this reporting period include:

- 7th Zebrafish Disease Models Conference, selected talk (abstract appended)
Title: Mechanistic insights into oncogenic glutamate receptor signaling in melanocytes and melanoma
- 22nd International Pigment Cell Conference, selected talk (abstract appended)
Title: Dual mechanisms combine to mediate regeneration of zebrafish melanocytes following injury
- 52nd Annual Meeting of The American Society of Dermatopathology, selected talk (abstract appended)
Title: The novel oncogene *GDF6* promotes melanoma cell survival
- PanAmerican Society for Pigment Cell Research, selected talk (abstract appended)
Title: Identifying *GDF6* as a novel pro-survival melanoma oncogene
- Tufts University, American Cancer Society seminar
Title: Understanding melanoma initiation using the zebrafish
- University of Massachusetts Medical School Hematology/Oncology seminar
Title: The BMP factor *GDF6* is a novel pro-survival melanoma oncogene
- MassBiologics research seminar
Title: The novel melanoma oncogene *GDF6* as a therapeutic target

Cell lines created during this reporting period include:

- *GDF6*-overexpressing melanoma cell lines
- *GDF6*-knockdown melanoma cell lines
- *SMAD1*-overexpressing melanoma cell lines
- *SMAD1*-knockdown melanoma cell lines
- *SMAD1DVD*-overexpressing cell lines

Zebrafish strains created during this reporting period include:

- Strains with loss-of-function mutations in *GDF6*
- Strains with *GDF6* overexpression in melanocytes

Publications include:

- Painter, C.A. and Ceol, C.J. (2014). Zebrafish as a platform to study tumor progression. *Methods in Molecular Biology*, 1176, 143-55.
- Iyengar, S., Kasheta, M. and Ceol, C.J. (2015). Poised regeneration of zebrafish melanocytes involves direct differentiation and concurrent replenishment of tissue-resident progenitor cells. *Developmental Cell*, 33, 631-43.

Personnel paid by research effort:

Craig Ceol, Ph.D.
Fang Liu, Ph.D.
Arvind Venkatesan

CONCLUSION:

In this reporting period we have obtained data that show *GDF6* is a new oncogene in melanoma. These data include gain-of-function data that show *GDF6* can promote tumor progression. Conversely, loss-of-function data indicate that *GDF6* is required for melanoma cell survival and, its loss abrogates tumor progression. Genetic epistasis and other data show that *GDF6* acts via the BMP signaling pathway to promote melanoma progression and melanoma cell survival. Our current hypothesis is that *GDF6* normally prevents terminal differentiation of melanocytes, thereby keeping cells in a progenitor-like state. Less differentiated progenitor-like cells are more apt to proliferate and support tumor progression.

These findings are important because *GDF6* represents a prime target for anti-melanoma therapy. *GDF6* encodes a secreted protein that is required for melanoma cell survival. Inhibition of *GDF6* has the potential to cause melanoma cell death and reduce tumor mass. Targeting *GDF6* protein could potentially be accomplished by inhibitors, such as monoclonal antibodies, that do not need to cross cell membranes. We are currently beginning to test this possibility by generating anti-*GDF6* antibodies.

REFERENCES:

1. C.J. Ceol, Y. Houvras, et al., The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset. *Nature*. **471**, 513-517 (2011).
2. C.A. Painter and Ceol, C.J., Zebrafish as a platform to study tumor progression. *Methods in Molecular Biology*, **1176**, 143-55 (2014).
3. M. Asai-Coakwell, C.F. French, et al., Incomplete penetrance and phenotypic variability characterize Gdf6-attributable oculo-skeletal phenotypes. *Human Molecular Genetics*. **18**, 1110-1121 (2009).
4. S. Tsukamoto, T. Mizuta, et al., Smad9 is a new type of transcriptional regulator in bone morphogenetic protein signaling. *Scientific Reports*. **4**, 7596 (2014).

APPENDICES:

Please see appended *curriculum vitae* for Dr. Ceol.
Please see appended meeting abstracts.

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Assistant Professor

Program in Molecular Medicine and Department of Molecular, Cell and Cancer Biology, University of Massachusetts
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Date prepared: August 1, 2015

EDUCATION

Yale University , New Haven, CT B.S./M.S. combined degree in Molecular Biophysics and Biochemistry Research Advisor: Dr. Lynne Regan	1989-1993
Massachusetts Institute of Technology , Cambridge, MA Ph.D. degree in Biology Research Advisor: Dr. H. Robert Horvitz	1995-2003
Massachusetts Institute of Technology , Cambridge, MA Postdoctoral Fellow, Department of Biology, HHMI Research Advisor: Dr. H. Robert Horvitz	2003-2004
Harvard Medical School, Children's Hospital Boston , Boston, MA Postdoctoral Fellow, Division of Hematology/Oncology, HHMI Research Advisor: Dr. Leonard I. Zon	2004-2008

PROFESSIONAL EXPERIENCE

Research Associate , Eli Lilly and Company Division of Bioproduct Development	1993-1995
Instructor , Harvard Medical School, Children's Hospital Boston, Division of Hematology/Oncology	2008-2009
Assistant Professor , University of Massachusetts Medical School Program in Molecular Medicine and Program in Cell Dynamics Department of Cancer Biology	2010-

HONORS AND AWARDS

Yale University: B.S./M.S. four-year degree, Molecular Biophysics and Biochemistry	1991-1993
Yale University Summer Study Grant	1991
Distinction in Molecular Biophysics and Biochemistry	1993
Massachusetts Institute of Technology: Koch Predoctoral Research Fellow	1999-2000
Children's Hospital Boston and Harvard Medical School: Damon Runyon Cancer Research Foundation Postdoctoral Fellowship	2005-2007
American Cancer Society Postdoctoral Fellowship (declined)	2005
Winner, Poster prize, Keystone Symposium, Santa Fe, NM <i>Advances in the Understanding and Treatment of Melanoma</i>	2006
Winner, Presentation prize, Harvard Stem Cell Institute Symposium	2008
Charles A. King Trust of The Medical Foundation Postdoctoral Fellowship	2008-2009
NIH Pathway to Independence Award (K99/R00), NIAMS	2009-2013
University of Massachusetts Medical School: Worcester Foundation for Biomedical Research Award	2011-2012
American Cancer Society Research Scholar Award	2012-2016
Kimmel Scholar Award	2013-2015

FUNDING

- Active:**
 RSG-12-150-01-DDC Research Scholar Award, American Cancer Society, Ceol (PI) Epigenetic determinants of melanoma initiation and maintenance.
 R01AR063850-01 NIH/NIAMS, Ceol (PI)
 Use of comparative oncogenomics to identify novel regulators of melanoma progression.
 CA120099 Dept of Defense Peer Reviewed Cancer Career Development Award, Ceol (PI)
 Uncovering the role of BMP signaling in melanocyte development and melanoma tumorigenesis.
 SKF-13-123 Kimmel Scholar Award, Ceol (PI)
 Mechanisms underlying melanoma initiation and maintenance.
 UL1TR000161 UMMS NHMPP Award, Ceol & Yang (PIs)
 GDF-6 blocking antibodies as cancer therapeutics.

- Concluded:**
 R00AR056899-04 Pathway to Independence Award, NIH/NIAMS, Ceol (PI)
 Identifying events and genetic regulators of melanoma progression
 P60016170000122 Worcester Foundation for Biomedical Research, Ceol (PI)
 Use of comparative genomics to identify oncogenes.
 Scientific Meeting Grant, The Company of Biologists, Ceol (PI)
 Zebrafish Disease Models 7 conference, Madison, WI, June 28-July 1, 2014

TEACHING AND MENTORING

- Teaching:**
- | | |
|---|------------|
| M.D./Ph.D. Research Tutorial, one discussion group (3hr). | 2010 |
| Ph.D. Summer RAPS (Reading, Analysis, Problem Solving paper review),
one discussion group (2hr). | 2010 |
| Cancer Biology, one lecture (2hr), one discussion group (2hr). | 2010- |
| Molecular Biology of the Cell Cycle, one lecture (0.5hr), one discussion group (2hr) | 2011, 2015 |
| Stem Cell and Regenerative Biology. Co-coordinator, two lectures and
discussion groups (4hr) plus organizational responsibilities. | 2011-2012 |
| RAPS, Block II (2hr). | 2011- |
| Topics in Molecular Medicine, one lecture and discussion group (2hr). | 2012 |
| MDP740 Developing solutions to research problems, lecture and discussion (2hr) | 2014 |

Advisory and supervisory responsibilities:

<u>Name</u>	<u>Position</u>	<u>Year(s)</u>	
Rajesh Vyas	Postdoctoral Fellow	2014-	
Ana Neto	Postdoctoral Fellow	2011-	NRSA Fellow, NCI
Fang Liu	Postdoctoral Fellow	2013	
Corrie Painter	Postdoctoral Fellow	2012-4	CRI Irvington Inst. Fellow
Sharanya Iyengar	Graduate Student	2010-	
James Neiswender	Graduate Student	2010-	
Arvind Venkatesan	Graduate Student	2011-	
Revati Darp	Graduate Student	2014-	
Alec Gramann	MD/PhD Student	2015-	
Tyler Frantz	MD/PhD Student	2015-	
Eli Freiman	Medical Student	2012	
Alysia Bryll	Rotating MD/PhD Student	2015	
Ciearra Smith	Rotating Graduate Student	2014	
Heather Kolpa	Rotating Graduate Student	2010	

Jennifer Maurer	Rotating Graduate Student	2010
James Ritch	Rotating Graduate Student	2010
Lin Lin	Rotating Graduate Student	2010
Justin Peter Hess	Undergrad. Student (WPI)	2012
Sukanya Murali	Undergrad. Student (Anna University – Chennai)	2013
Brittney Logan	Undergrad. student (W. New England University)	2013

Dissertation committees:

Shawna Guillemette, UMass Medical School, Cancer Biology Program
Tomoko Tabuchi, UMass Medical School, Interdisciplinary Graduate Program
David Driscoll, UMass Medical School, Cancer Biology Program
Anna Malinkevich, UMass Medical School, Interdisciplinary Graduate Program
Cheng Chang, UMass Medical School, Cancer Biology Program (Chair)
Nomeda Girnius, UMass Medical School, Cancer Biology Program
Lin Lin, UMass Medical School, Cancer Biology Program (Chair)
James Ritch, UMass Medical School, Interdisciplinary Graduate Program
Nicola Kearns, UMass Medical School, Interdisciplinary Graduate Program

Qualifying examination committees:

Christopher Clark, UMass Medical School, Neuroscience Program
Caitlin Fogarty, UMass Medical School, MD/PhD Program
Nomeda Girnius, UMass Medical School, Interdisciplinary Graduate Program
Chien-Min Hung, UMass Medical School, Interdisciplinary Graduate Program
James Ritch, UMass Medical School, Interdisciplinary Graduate Program
Lin Lin, UMass Medical School, Cancer Biology Program (Chair)
Ly-She Ee, UMass Medical School, Interdisciplinary Graduate Program
Shubham Dutta, UMass Medical School, Interdisciplinary Graduate Program
Nicola Kearns, UMass Medical School, Interdisciplinary Graduate Program
Hsi-Ju Chen, UMass Medical School, Interdisciplinary Graduate Program
Nicholas Panzarino, UMass Medical School, Cancer Biology Program (Chair)

SERVICE

University of Massachusetts Medical School and local:

Sherman Center Labs NTI/GTC/CVC/Diabetes Focus Group	2010
Diabetes and Endocrinology Research Center (grant reviewer, ad hoc)	2011
AP Biology High School Outreach Program (host)	2011-
University of Massachusetts Medical School Convocation (Dinner and Dialogue event speaker and panelist)	2011
University of Massachusetts Medical School visit of Young President's Organization & World President's Organization (speaker)	2011
University of Massachusetts Medical School Development Council meeting (speaker)	2012
University of Massachusetts Medical School BARG Organization (speaker)	2012
LCME accreditation of University of Massachusetts Medical School (Junior Faculty cohort)	2012
University of Massachusetts Chancellor's Review (Faculty Review Committee)	2012
Wachusett High School Science Seminar	2012
University of Massachusetts Medical School Science to Trades Seminar	2013
WSRS interview w/ Greg Byrne in support of UMMS Cancer Walk	2013
UMMS Development Office – lab tours with donor groups	2013-
MassAHEC Network Frontiers in Science Seminar	2014
NIH BEST Award Focus Group	2014
Hudson Hoagland Society annual meeting (speaker)	2014
WSRS interview W/ Jordan Levy in support of UMMS Cancer Walk	2014

University of Massachusetts Medical School Media Day (speaker)	2014
UMMS Communications Office – ‘Here for a Reason’ campaign	2014
Cancer Walk Kickoff Breakfast (panelist)	2015

Referee for journals:

Molecular and Cellular Oncology – Peer Review Board 2014-
 PLoS Genetics – ad hoc 2009-
 Proceedings of the National Academy of Sciences USA – ad hoc 2009-
 Molecular and Cellular Biology – ad hoc 2010-
 PLoS Biology – ad hoc 2011-
 FASEB Journal – ad hoc 2012-
 Experimental Cell Research – ad hoc 2012-
 Genome Research – ad hoc 2012-
 Journal of Investigative Dermatology – ad hoc 2012-
 Journal of Visualized Experiments – ad hoc 2013-
 Cell Death and Differentiation – ad hoc 2013-
 Disease Models and Mechanisms – ad hoc 2013-
 Cell Death and Disease – ad hoc 2014-

Grant review and study section service:

Children's Tumor Foundation – 2013-
 National Centre for the Replacement, Refinement and Reduction of Animals in Research (Ad Hoc Reviewer) -
 2010
 University of Massachusetts Medical School Diabetes and Endocrinology Research Center (Ad Hoc Reviewer) -
 2010
 Association for International Cancer Research (Ad Hoc Reviewer) - 2011
 NIH, Cancer Genetics Study Section (CG) (Ad Hoc Reviewer) - 2012
 Medical Research Council (United Kingdom) (Ad Hoc Reviewer) - 2013, 2015
 NIH, Genes, Genomes and Genetics Special Emphasis Review panel ZRG1 GGG-E - 2015
 MSKCC-CCNY U54 Translational Research (Ad Hoc reviewer) - 2015

Society memberships:

Society for Melanoma Research, 2009-
 American Society for Cell Biology, 2012-
 American Association for Cancer Research, 2012-
 Zebrafish Disease Models Society, 2014-
 Pan-American Society for Pigment Cell Research, 2014-

Meetings and community service:

Co-organizer, Zebrafish Disease Models 7, Madison, WI, 2014
 Co-chair, Cancer Working Group, Zebrafish Disease Models Society, 2014-
 Session Chair, Society for Developmental Biology Northeast meeting, Woods Hole, MA, 2015

PUBLICATIONS

Original reports:

1. **Ceol, C.J.** and Horvitz, H.R. (2001). *dpl-1* DP and *efl-1* E2F act with *lin-35* Rb to antagonize Ras signaling in *C. elegans* vulval development. *Mol. Cell.* 7, 461-73.
 ‡ This paper is highlighted by the Faculty of 1000.
2. Thomas, J.H.*, **Ceol, C.J.***, Schwartz, H.T. and Horvitz, H.R. (2003). New genes that interact with *lin-35* Rb to negatively regulate the *let-60 ras* pathway in *Caenorhabditis elegans*. *Genetics.* 164, 135-51.
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4. **Ceol, C.J.**, Stegmeier, F., Harrison, M.M. and Horvitz, H.R. (2006). Identification and classification of genes that act antagonistically to *let-60* Ras signaling in *Caenorhabditis elegans* vulval development. *Genetics.* 173, 709-26.

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 9. Freeman, J.L., **Ceol, C.J.**, Feng, H., Langenau, D.M., Belair, C., Stern, H.M., Song, A, Paw, B.H., Look, A.T., Zhou, Y., Zon, L.I. and Lee, C. (2009). Construction and application of a cytogenetically-validated zebrafish-specific array CGH platform. *Genes Chromosomes Cancer*, 48, 155-70.
 10. North, T.E., Goessling, W., Peeters, M., Li, P., **Ceol, C.J.**, Lord, A.M., Weber, G.J., Harris, J., Cutting, C.C., Huang, P., Dzierzak, E., Zon, L.I. (2009). Hematopoietic stem cell development is dependent on blood flow. *Cell*, 137, 436-48.
 11. **Ceol, C.J.***, Houvras, Y.*, Jane-Valbuena, J., Bilodeau, S., Orlando, D., Battisti, V., Fritsch, L., Lin, W., Hollmann, T.J., Ferré, F., Bourque, C., Burke, C., Turner, L., Uong, A., Johnson, L.A., Beroukhim, R., Mermel, C., Loda, M., Ait-Si-Ali, S., Garraway, L., Young R.A. and Zon, L.I. (2011). The *SETDB1* histone methyltransferase is recurrently amplified in and accelerates melanoma. *Nature*, 471, 513-7.
 12. Richardson, J., Zeng, Z., **Ceol, C.J.**, Jackson, I.J., Patton, E.E. (2011). *BRAF*^{V600E} nevi regenerate from an undifferentiated precursor population in zebrafish. *Pigment Cell Melanoma Research*, 24, 378-81.
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- ‡ This paper is highlighted by the Faculty of 1000.
14. Iyengar, S., Houvras, Y. and **Ceol, C.J.** (2012). Screening for melanoma modifiers using a zebrafish autochthonous tumor model. *Journal of Visualized Experiments*, 69, e50086.
 15. Painter, C.A. and **Ceol, C.J.** (2014). Zebrafish as a platform to study tumor progression. *Methods in Molecular Biology*, 1176, 143-55.
 16. Iyengar, S., Kasheta, M. and **Ceol, C.J.** (2015). Poised regeneration of zebrafish melanocytes involves direct differentiation and concurrent replenishment of tissue-resident progenitor cells. *Developmental Cell*, 33, 631-43.
- ‡ Previewed in Kang, J., Karra, R. and Poss, K. (2015) Back in black. *Developmental Cell*, 33, 623-4.

Reviews and commentary:

1. **Ceol, C.J.**, Pellman D. and Zon, L.I. (2007). APC and colon cancer: two hits for one. *Nat. Med.* 13, 1286-7.
2. **Ceol, C.J.***, Houvras, Y.*, White R.M.* and Zon, L.I. (2008). Melanoma biology and the promise of zebrafish. *Zebrafish* 5, 247-55.
3. **Ceol, C.J.** (2011). Acta Eruditorum: Certain genes accelerate melanoma development. *Dermatology World* 21, 11-12.

Cover art:

1. **Ceol, C.J.***, Houvras, Y.*, Jane-Valbuena, J., Bilodeau, S., Orlando, D., Battisti, V., Fritsch, L., Lin, W., Hollmann, T.J., Ferré, F., Bourque, C., Burke, C., Turner, L., Uong, A., Johnson, L.A., Beroukhim, R., Mermel, C., Loda, M., Ait-Si-Ali, S., Garraway, L., Young R.A. and Zon, L.I. (2011). The *SETDB1* histone methyltransferase is recurrently amplified in and accelerates melanoma. *Nature*, 471, 513-7.
2. Iyengar, S., Kasheta, M. and **Ceol, C.J.** (2015). Poised regeneration of zebrafish melanocytes involves direct differentiation and concurrent replenishment of tissue-resident progenitor cells. *Developmental Cell*, 33, 631-43.

ORAL PRESENTATIONS

Meeting presentations:

East Coast <i>C. elegans</i> Meeting, Boston, MA	1998
International <i>C. elegans</i> Meeting, Madison, WI	1999
East Coast <i>C. elegans</i> Meeting, Durham, NH	2002
Keystone Symposium, <i>Advances in the Understanding and Treatment of Melanoma</i> , Santa Fe, NM	2006
Gordon Conference, <i>Cancer Models and Mechanisms</i> , Les Diablerets, Switzerland	2008
8th International Conference on Zebrafish Development and Genetics, Madison, WI	2008
Harvard Stem Cell Institute Research Symposium, Boston, MA	2008
9th International Conference on Zebrafish Development and Genetics, Madison, WI	2009
3rd Zebrafish Disease Models Conference, Boston, MA	2010
Connecticut Valley Zebrafish Meeting, Middletown, CT	2010
Gordon Conference, <i>Cancer Genetics and Epigenetics</i> , Ventura, CA	2011
Biotechcellence 2012 National Technical Symposium Anna University, Chennai, India (via videoconference)	2012
10th International Conference on Zebrafish Development and Genetics, Madison, WI (workshop co-coordinator)	2012
International Federation of Pigment Cell Societies, Pigment Cell Development Workshop, Edinburgh, UK	2013
5th European Melanoma Conference, <i>Basic and clinical research join forces to defeat melanoma</i> , Marseille, France	2013
6th Zebrafish Disease Models Conference, Murcia, Spain	2013
7th Zebrafish Disease Models Conference, Madison, Wisconsin (in place of maternity leave postdoc Ana Neto)	2014
22nd International Pigment Cell Conference, <i>Bringing colours to life</i> , Singapore	2014
52nd Annual Meeting of The American Society of Dermatopathology, San Francisco, CA	2015
PanAmerican Society for Pigment Cell Research Conference, Irvine, CA	2015

Invited seminar presentations:

Hubrecht Institute, Utrecht, Netherlands Cancer Genomics and Developmental Biology Programme Seminar	2008
Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA Whitehead Seminar Series for High School Teachers: Controlling Genes	2008
Providence College, Providence, RI Biology Department Seminar	2011
UMass Medical School, Worcester, MA Cutaneous Tumor Board, Pathology Department	2011
University of Rochester Medical Center, Rochester, NY Biomedical Genetics Department Seminar	2011
Quinsigamond Dermatological Society, Worcester, MA Grand Rounds	2011
Carnegie Institution, Baltimore, MD Department of Embryology Seminar	2012
National Institutes of Health, Bethesda, MD NIH Comparative Biomedical Scientist Program Symposium	2012
University of Massachusetts Medical School, Worcester, MA Cancer Biology Retreat	2012
Assumption College, Worcester, MA Seminar in Life Sciences	2012
University of Massachusetts Medical School, Worcester, MA Microbiology and Physiological Systems Department Seminar	2013
Tufts University School of Medicine, Boston, MA Molecular Physiology and Pharmacology Retreat (Keynote)	2013
Centro Andaluz de Biología del Desarrollo, Seville, Spain CABD Institute Seminar	2013
University of Michigan, Ann Arbor, MI	2014

Molecular, Cellular and Developmental Biology Seminar University of Massachusetts, Dartmouth, MA	2014
Biology and Bioengineering Seminar Tufts University, Medford, MA	2015
American Cancer Society Relay for Life Seminar University of Massachusetts Medical School, Worcester, MA Division of Hematology/Oncology Grand Rounds	2015

Mechanistic insights into oncogenic glutamate receptor signaling in melanocytes and melanoma

Ana Neto and Craig Ceol

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Glutamate signaling, which is important in the central nervous system and in glial cell function, has recently been shown to have a role in melanoma progression. Human melanoma exome sequencing studies have identified activating mutations in metabotropic glutamate receptor 3 (GRM3). We hypothesize that altered glutamate signaling affects the development and function of melanocytes, endowing these cells with properties important for melanoma progression. Accordingly, understanding the role of glutamate signaling in melanocytes may inform how dysregulation of glutamate signaling is involved in melanoma progression. To test our hypothesis we use the miniCoopR assay, in which transgene-bearing melanocytes are derived in a *mitfa(lf)* background. Using this assay, we have determined how oncogenic GRM3 variants affect developing melanocytes and impact tumor formation. In embryonic melanocytes oncogenic GRM3 mutants disrupt trafficking of melanosomes, the pigment-producing organelles, whereas wild-type GRM3 does not. These and other data indicate that oncogenic GRM3 variants dysregulate cyclic AMP (cAMP) signaling, a heretofore unknown role for these oncogenes. Extending our analyses to tumors, we have found that expression of oncogenic GRM3 affects melanoma onset. These and additional data suggest that altered cAMP signaling can impact melanoma progression. Recent data have implicated defective cAMP signaling in the melanoma susceptibility of red-haired, fair-skinned individuals. Our data support the notion that disrupted cAMP signaling is a more pervasive contributor to melanoma, including in individuals that have incurred GRM3 mutations.

Dual mechanisms combine to mediate regeneration of zebrafish melanocytes following injury

Sharanya Iyengar, Craig Ceol

Program in Molecular Medicine and Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA

Melanocytes, which can be lost during hair graying, injury and disease-related depigmentation, are replenished in mammals by resident stem cells. To gain insight into melanocyte regeneration we set out to identify whether melanocyte stem cells are present in adult zebrafish and how such cells might reconstitute the pigment pattern following injury. Using a targeted cell ablation approach we determined that *mitfa* is expressed not only in differentiated melanocytes but also in the cells that mediate melanocyte regeneration. When *mitfa*-positive cells are selectively ablated no melanocyte regeneration occurs. However, when ablation is performed in a *p53*-deficient background, melanocyte regeneration occurs, suggesting that death of the cells that mediate regeneration is dependent on *p53*. We then used *mitfa*-positivity to perform lineage-tracing experiments and assay whether unpigmented *mitfa*-expressing cells have stem cell properties. During regeneration, *mitfa*-positive cells can divide asymmetrically with one daughter cell differentiating and the other daughter remaining uncommitted; these are melanocyte stem cell divisions. In addition, some *mitfa*-positive cells directly differentiate during regeneration. Taken together, these data indicate that multiple mechanisms are used to re-establish pigmentation following injury and enable regeneration following subsequent rounds of ablation. We have used reporter assays and drug studies to assess whether pathways important for melanocyte development are also involved in regeneration. We found that Wnt signaling gets turned on during melanocyte regeneration and that Wnt inhibition after ablation of differentiated melanocytes delays regeneration. These studies have established a system by which regeneration can be traced with single-cell resolution and perturbations to regeneration analyzed in exquisite detail.

The novel oncogene *GDF6* promotes melanoma cell survival

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The prevalence of *BRAFV600E* in nevi indicates that this mutation is not sufficient to cause melanoma. To identify new melanoma genes that could cooperate with *BRAFV600E*, we searched for abnormalities shared in both human melanomas and in a zebrafish *BRAFV600E*-driven melanoma model. We hypothesized that these conserved abnormalities would be enriched for genes that affect melanoma progression. In these analyses, we identified the *GDF6* gene, which encodes a member of the bone morphogenetic protein (BMP) family. *GDF6* genes in humans and zebrafish were recurrently copy number amplified in melanoma, and expression of the *GDF6* gene was observed in human and zebrafish melanomas but absent from normal melanocytes in both species. In functional analyses, overexpression of *GDF6* accelerated melanoma progression, whereas knockdown of *GDF6* in cultured A375 cells compromised melanoma formation in xenotransplantation assays. Knockdown of *GDF6* caused programmed cell death, which was rescued by an activated variant of the SMAD1 transcription factor, indicating that *GDF6* acts through the canonical BMP signaling pathway. Strikingly in tissue sections, GDF6 protein was readily detectable in more than 90% of melanomas, but was absent from melanocytes in normal adjacent skin. BMP pathway activity was likewise apparent in melanomas in a pattern that overlapped with GDF6 staining. Taken together, these data indicate that *GDF6* is a new melanoma oncogene that promotes melanoma cell survival and can be therapeutically targeted to induce melanoma cell death.

Identifying GDF6 as a novel pro-survival melanoma oncogene

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⁴ Sanford Burnham Medical Research Institute, Orlando, FL, USA

To identify genes involved in tumor progression we defined regions of recurrent copy number variation in zebrafish melanomas and compared these regions to ones recurrently altered in human melanomas. In the set of genes that were recurrently amplified in both species we found the BMP factor GDF6. In analyses of both zebrafish and humans, GDF6 mRNA and protein were upregulated in melanomas as compared to normal melanocytes. In functional assessments, we found that overexpression of GDF6 accelerated melanoma onset in zebrafish and mouse xenotransplantation assays. Furthermore, knockdown of GDF6 in melanoma cell lines led to apoptotic cell death in culture *in vitro* and in *GDF6*-deficient tumors *in vivo*. Addition of recombinant GDF6 protein to the media prevented melanoma cells from undergoing GDF6 shRNA-induced apoptosis, suggesting that GDF6 acts as a secretory factor in aiding melanoma cell survival. GDF6, like other BMP factors, is predicted to signal through SMAD1/5/8 transcription factors, and similar defects were observed when SMAD1 was knocked down. To further define the relationship between GDF6 and SMAD1 in melanoma, GDF6 knockdown was performed in cells expressing a constitutively active SMAD1 variant. This variant rescued the death caused by GDF6 knockdown, suggesting that, at least in part, GDF6 acts through SMAD1 to promote melanoma cell survival. These data establish a role for BMP signaling in melanoma and identify a novel secretory factor, GDF6 that mediates this role. Discovery of this novel secretory factor that is present in a majority of human melanomas provides an excellent therapeutic target.

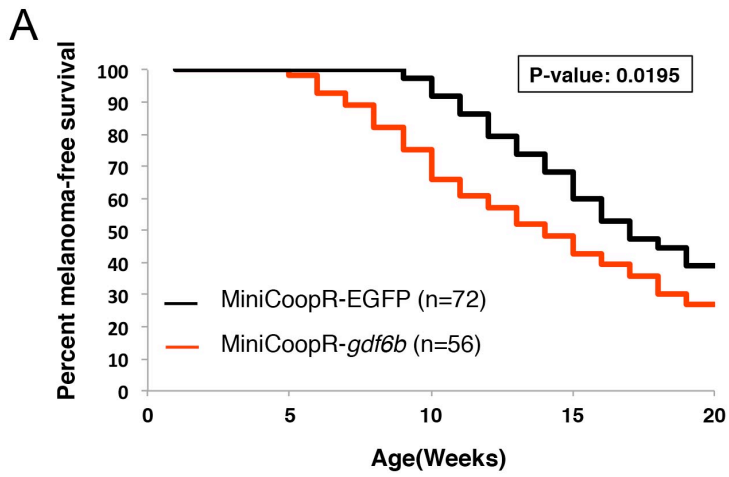
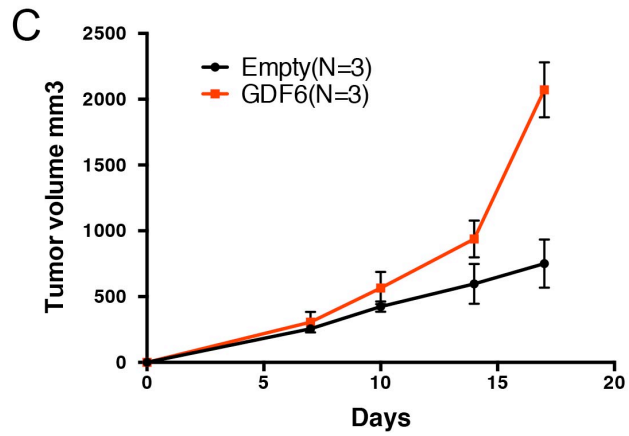
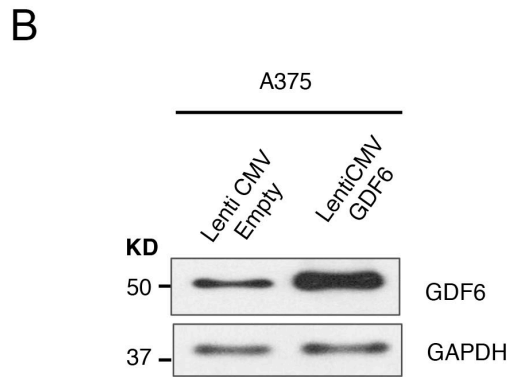
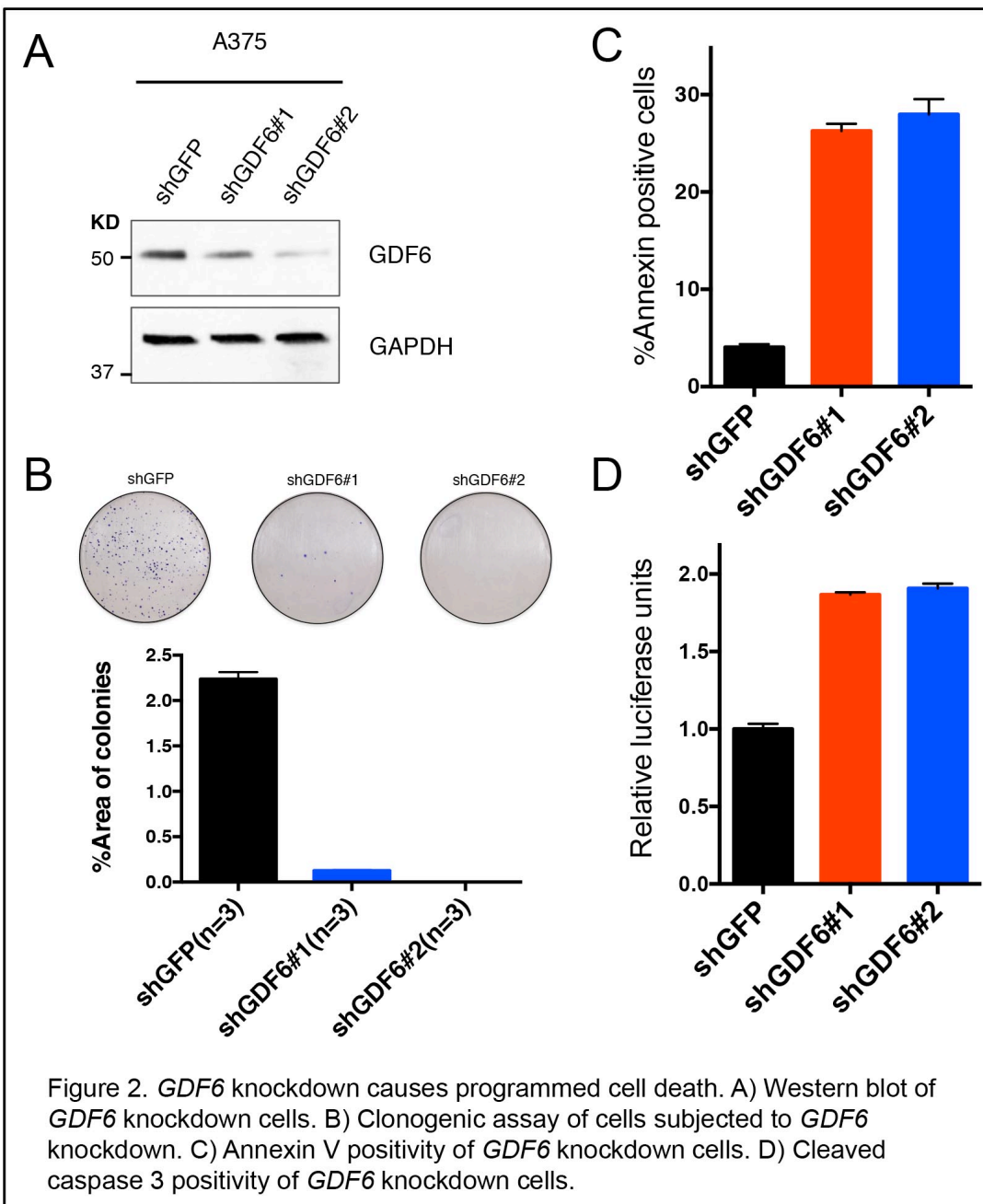


Figure 1. *GDF6* expression accelerates melanoma onset. A) Expression of *gdf6b* accelerates melanoma onset. In this assay melanocytes expressing the zebrafish *GDF6* ortholog *gdf6b* or *EGFP* were reconstituted in a *BRAF^{V600E}*-positive and *p53*-negative background. B) *GDF6* expression in control and *GDF6* overexpression cell lines. C) Cells that overexpress *GDF6* have accelerated melanoma onset in xenotransplantation.





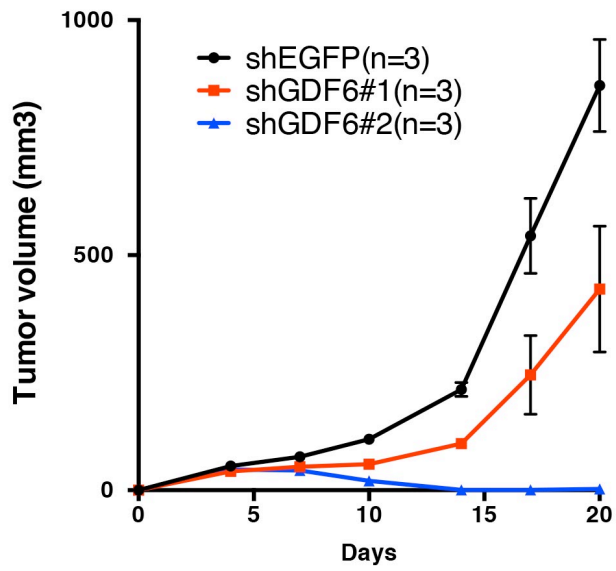


Figure 3. *GDF6* knockdown abrogates tumor progression.

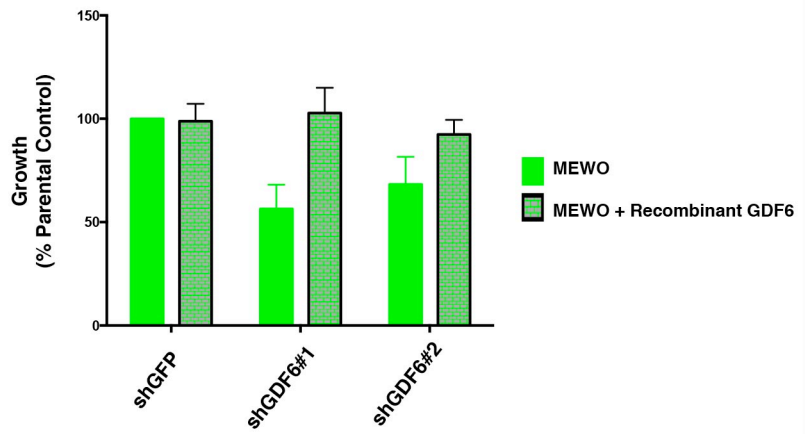


Figure 4. Recombinant GDF6 rescues the proliferation defect caused by *GDF6* knockdown. Experiments were performed with MeWo melanoma cells, and recombinant GDF6 protein was added to culture media for rescue experiments.

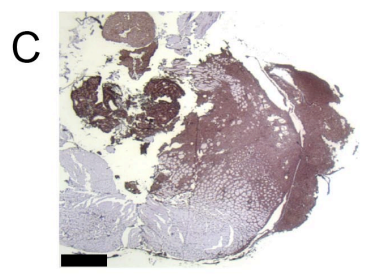
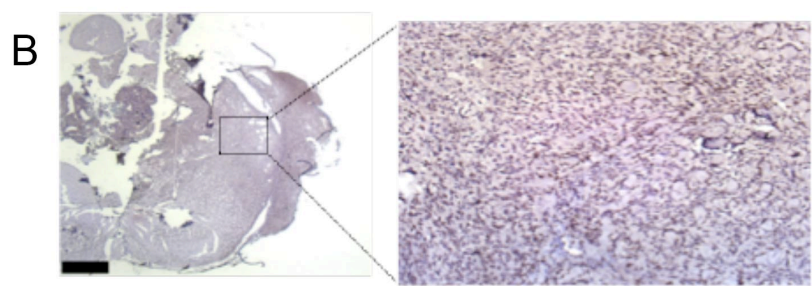
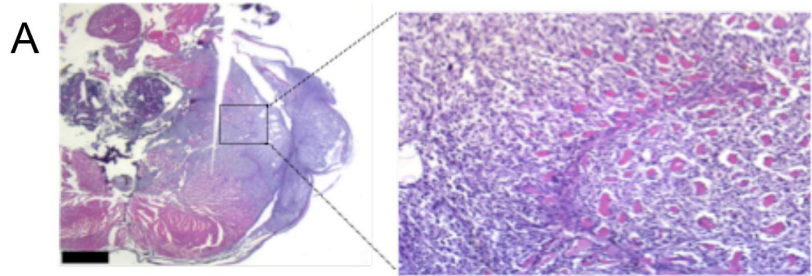
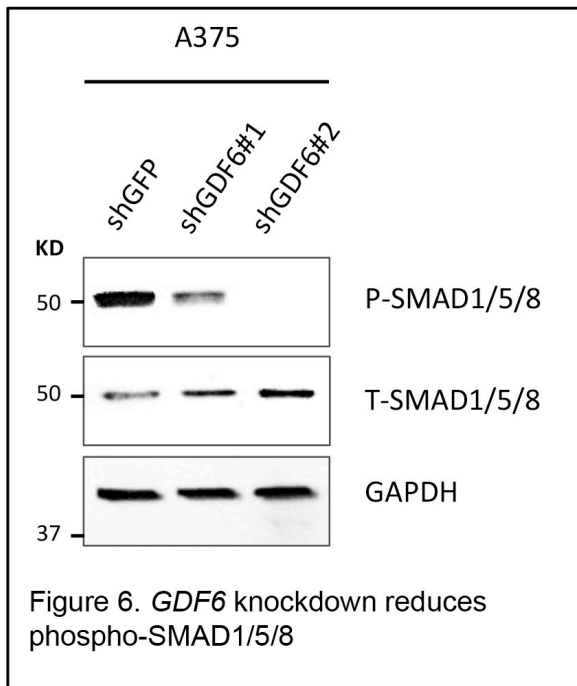
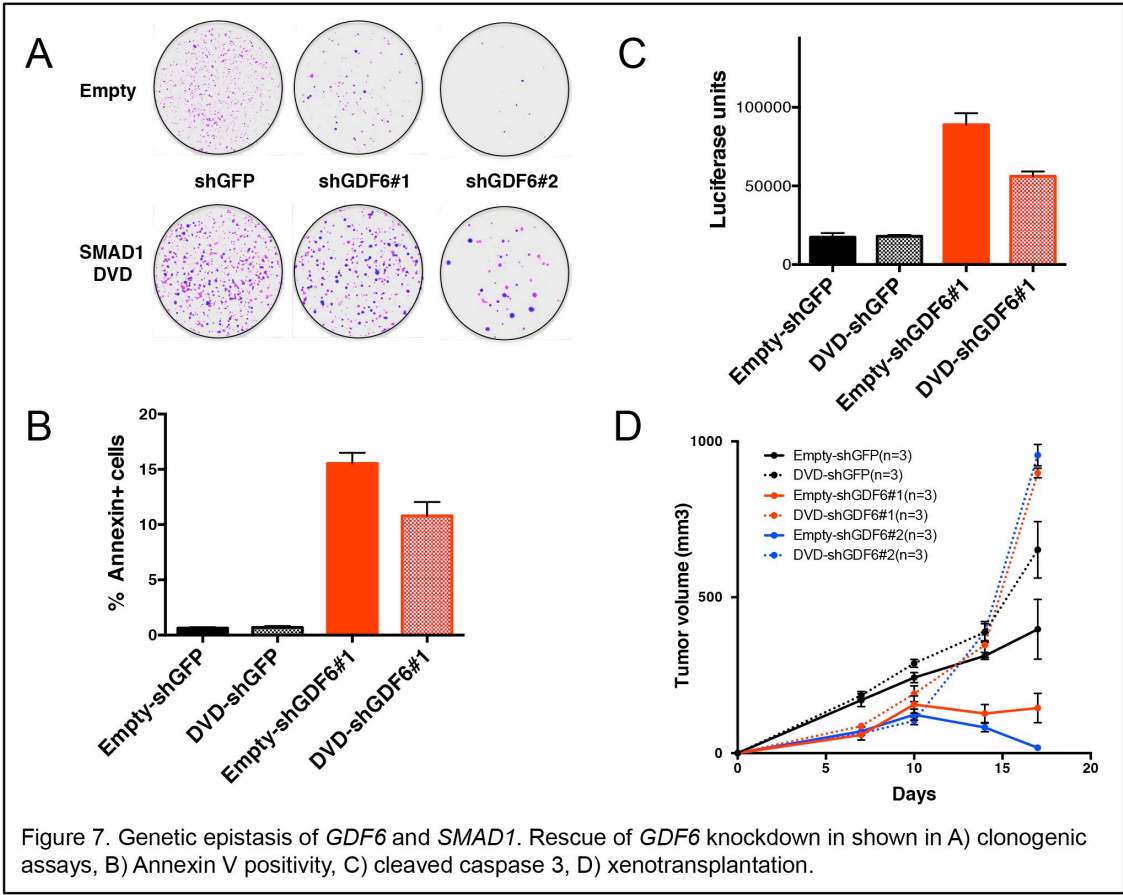


Figure 5. High levels of GDF6 and phospho-SMAD1/5/8 in zebrafish. A) H+E staining of zebrafish melanoma. B) phospho-SMAD staining. C) GDF6 staining





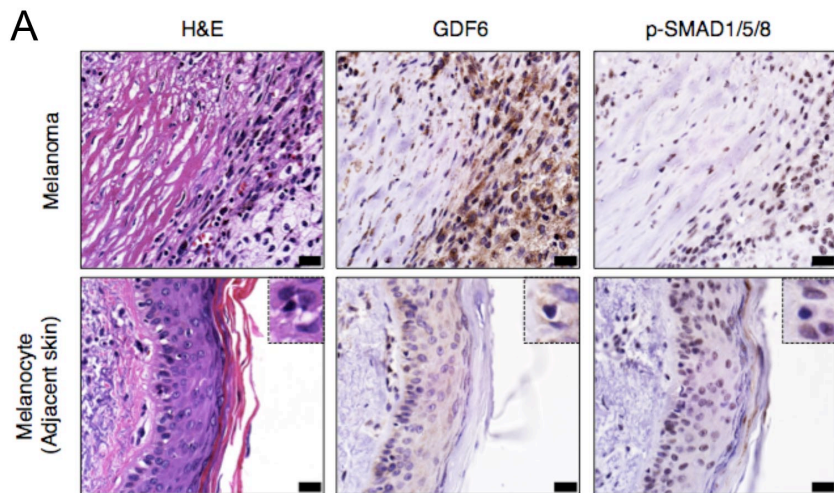


Figure 8. GDF6 and SMAD1 staining in human melanomas. A) H+E stains are shown (left). Robust GDF6 (middle) and phospho-SMAD1/5/8 expression are typically found in human melanomas but not normal melanocytes. B) Quantification of staining.

