

Washington University School of Medicine

Digital Commons@Becker

Open Access Publications

2018

Feeding amount significantly alters overt tumor onset rate in a zebrafish melanoma model

Vadim Grigura

Washington University School of Medicine in St. Louis

Megan Barbier

Washington University School of Medicine in St. Louis

Anna P. Zarov

Washington University in St. Louis

Charles K. Kaufman

Washington University School of Medicine in St. Louis

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Please let us know how this document benefits you.

Recommended Citation

Grigura, Vadim; Barbier, Megan; Zarov, Anna P.; and Kaufman, Charles K., "Feeding amount significantly alters overt tumor onset rate in a zebrafish melanoma model." *Biology Open*. 7, 1. (2018).

https://digitalcommons.wustl.edu/open_access_pubs/6554

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.

RESEARCH ARTICLE

Feeding amount significantly alters overt tumor onset rate in a zebrafish melanoma model

Vadim Grigura¹, Megan Barbier¹, Anna P. Zarov² and Charles K. Kaufman¹

ABSTRACT

The manner in which zebrafish are fed may have important impacts on the behavior of disease models. We examined the effect of different feeding regimens on the rate of overt melanoma tumor onset in a *p53/BRAF*-dependent model, a commonly used read-out in this and many other cancer models. We demonstrate that increased feeding leads to more rapid melanoma onset. The ability to modulate overt tumor onset rates with this regimen indicates additional flexibility to 'tune' the system to more quickly generate tumors for study and to begin to address questions related to cancer metabolism using the zebrafish model.

KEY WORDS: Melanoma, Cancer models, Metabolism, Obesity, Caloric restriction

INTRODUCTION

Genetic models of cancer are dependent on defined driver oncogenes and tumor suppressors, thus creating a homogeneous population in which to study the effect of chosen perturbations on cancer formation. One limitation of such genetically engineered adult tumor models is that there may be a delay to onset of detectable tumors, which slows analysis, adds costs for housing, and can fill available facility space. The long-recognized association between food/caloric intake and longevity/overall health, including rates of cancer, in many organisms (Ravussin et al., 2015; Mattison et al., 2017; Colman et al., 2014; Klass, 1977; Bross et al., 2005; Weindruch and Walford, 1982) led us to wonder if altered feeding of a favored zebrafish melanoma model (i.e. *p53/BRAF* model) would alter the rate of grossly detectable tumor onset (Patton et al., 2005; Ceol et al., 2011; White et al., 2011; Lian et al., 2012; Kaufman et al., 2016).

RESULTS AND DISCUSSION

Our zebrafish facility employs an aggressive feeding protocol using Tecniplast Tritone robotic feeders throughout the fish life-span (Fig. 1A), allowing for rapid rearing of zebrafish as our standard protocol. The use of the Tritone feeders also allows for precise and simple manipulation of daily feeding programs. Parental fish homozygous for the melanoma-inducing *p53/BRAF* mutations were

incrossed, and 50 embryos were placed per 10 cm petri dish in E3 buffer until 6 days postfertilization (dpf) (Fig. 1A). Some parental fish carried the *Tg(crestin:EGFP)* transgene, but this was not analyzed for this study (Kaufman et al., 2016). Each dish was then reared in a 3.5 liter tank with excess rotifers beginning at 6 dpf until 10-14 days of life. Developing juvenile zebrafish were then fed via Tritone robot increasing aliquots of GEMMA pellet (Skretting)±rotifer culture (Fig. 1A; Table S1). Importantly, to minimize any 'jackpot' effects of single tanks, all juvenile zebrafish born on the same day were mixed during 4-5 weeks of life, and then randomly redistributed with 25 individuals per 3.5 liter tank. These tanks were then moved from the nursery to the main system during the sixth week of life and assigned a feeding label providing four (4X), two (2X), and one (1X) time daily feeding of the same weight (60 mg) of GEMMA pellet per feeding (Fig. 1A).

As previously described, visual inspection of *p53/BRAF* zebrafish for raised lesions identifies melanoma tumors reproducibly, and this approach has been used to establish the role of multiple factors in modifying melanomagenesis (Ceol et al., 2011; Lian et al., 2012). Thus, each cohort of zebrafish was scored for melanoma onset every 2 weeks beginning at 9-10 weeks of age. The total number of animals in each tank was kept constant by replacing any tumor-bearing zebrafish, which were removed upon identification of a tumor, with similarly aged *Casper* or *Na* zebrafish. As shown in Fig. 1B, the rate of detectable melanoma was significantly increased in three independent cohorts of *p53/BRAF* melanoma-prone zebrafish fed four times daily (4X, median onset 180, 162 and 187 days) as compared to those fed two (median onset 222, 230 and 223 days) or one time daily (median not reached in any cohort). These were highly significant differences comparing the four versus two times daily ($P<0.0001$, $P<0.0001$ and $P<0.0013$) and four versus one time daily ($P<0.0001$ in each cohort) schedules. There was also a trend of quicker onset in the two versus one daily groups, although not significant in all cohorts ($P=0.22$, $P=0.0458$ and $P=0.0676$). As all cohorts were fed our standard diet in the nursery, we did not intensively investigate effects on sexual maturity. The appearance of the melanomas in all groups at the time of tumor identification was qualitatively similar, and in this study, we did not track the behavior of established tumors in the different feeding groups. We measured a snapshot of size [(i.e. snout to tail, SL, length (Parichy et al., 2009))] of zebrafish in Cohort 1 at 155 and 211 days of life and found stable differences in size between the 4X and 2X/1X daily feedings (Fig. 1C,D).

Overall, our data reveal a significant modulation of gross melanoma tumor onset by the amount of feeding of adult zebrafish. Published datasets fit the consensus that the *p53/BRAF* melanoma model [e.g. median onset of ~35 weeks (Neiswender et al., 2017) and not reached at 18 months (Lister et al., 2014)] typically produces tumors more slowly than the *p53/BRAF/Na/MiniCoopR* model [e.g. median onset of ~19 weeks for controls (Lian et al., 2012; Ceol et al., 2011)]. In this study, aggressive

¹Division of Medical Oncology, Department of Medicine and Department of Developmental Biology, Washington University in Saint Louis, 4518 McKinley Ave., CB 8069, St. Louis, MO 63110, USA. ²School of Arts and Sciences, Washington University in Saint Louis, 1 Brookings Drive, St. Louis, MO, 63130, USA.

*Author for correspondence (ckkaufman@wustl.edu)

 C.K.K., 0000-0003-3122-1677

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

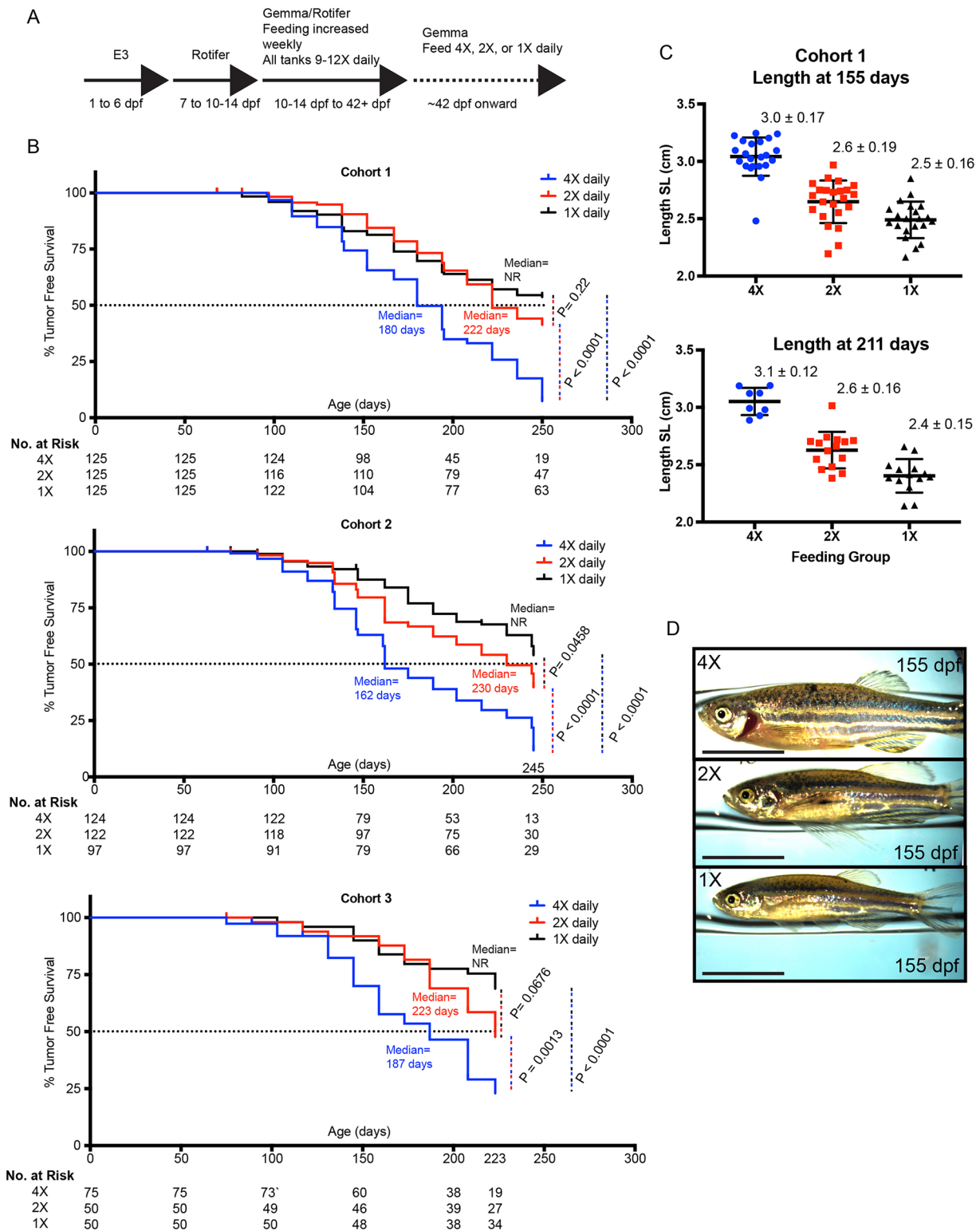


Fig. 1. Feeding amount alters melanoma tumor onset in zebrafish *p53/BRAF* model. (A) Feeding paradigm used to rapidly rear *p53/BRAF* melanoma-prone zebrafish. (B) Gross tumor onset reported as Kaplan–Meier curves for percent (%) tumor-free survival at the indicated age (dpf). Number of zebrafish at risk are reported at 50 day intervals based on the most proximal prior observation. Reported *P* values are based on the Log-rank (Mantel-Cox) Test as determined by Prism software where curves were generated. NR, not reached. (C) Zebrafish length at the indicated times for various adult feeding regimens. SL, snout to caudal peduncle length, is reported in centimeters with mean±standard deviation. (D) Representative images of *p53/BRAF* zebrafish at 155 dpf in each feeding group. Scale bar: 1 cm.

rearing in the nursery and 4X daily feeding of adults achieved median onset of ~25 weeks, approaching the speed of the *MiniCoopR* system (Fig. 1B). In humans and in mouse models,

there does appear to be a link between obesity and melanoma formation and aggressiveness, consistent with potential modulation of melanoma behavior by obesity-linked metabolic changes

(Clement et al., 2017). A number of studies have also found an important role for diet composition (i.e. high-fat content, antioxidants) and caloric intake on melanoma metastasis and growth, but in the setting of murine transplantation models (Piskounova et al., 2015; Erickson et al., 1979; Erickson, 1984; Rofe et al., 1985; Ershler et al., 1986; Malvi et al., 2014; Xia et al., 2017). Perhaps most intriguingly in this study, our current feeding paradigm supports the possibility of applying the molecular genetic toolbox offered by the zebrafish to rapid mechanistic studies of the relationship of diet, caloric intake, and metabolism to *de novo* melanoma tumor formation.

MATERIALS AND METHODS

Zebrafish husbandry

Zebrafish of the *p53/BRAF/crestin:EGFP* genotype (Patton et al., 2005; Kaufman et al., 2016) were maintained in a Tecniplast system with Tritone robotic feeding system at 28.5°C under standard housing protocols as per Institutional Animal Care and Use Committee (IACUC) regulations. GEMMA Micro 150 and 300 pellets were from Skretting, a Nutreco company. Details of the feeding protocol are provided in Fig. 1A and Table S1, and all experiments were performed under IACUC-approved protocols.

Scoring of zebrafish for tumor formation

Every 2 weeks beginning at 9–10 weeks of age, zebrafish were briefly separated, five fish per holding tank, for counting, and then individually observed for presence of raised lesions, which are indicative of melanoma tumor formation as extensively described (Patton et al., 2005; Ceol et al., 2011; Kaufman et al., 2016). Those fish with melanoma tumors were removed and replaced with similarly age-matched *Casper* or *Nacre* fish, allowing for easy identification of ‘place-holder’ fish. Tumor onset data were entered into GraphPad Prism® to generate survival curves with *P* values based on the Log-rank (Mantel-Cox) Test.

Acknowledgements

We thank members of the Washington University Zebrafish Core Facility, including Steve Canter and John Englehard. We are grateful to Eva Kramer for helpful comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.K.K.; Methodology: C.K.K.; Software: C.K.K.; Validation: C.K.K., V.G., M.B., A.P.Z.; Formal analysis: C.K.K., V.G.; Investigation: C.K.K., V.G., M.B., A.P.Z.; Resources: C.K.K., V.G., M.B., A.P.Z.; Data curation: C.K.K., V.G.; Writing – original draft: C.K.K.; Writing – review & editing: C.K.K., V.G., M.B., A.P.Z.; Visualization: C.K.K., V.G.; Supervision: C.K.K.; Project administration: C.K.K.; Funding acquisition: C.K.K.

Funding

This research was supported in part by the Cancer Research Foundation (Young Investigator Award to C.K.K.).

Supplementary information

Supplementary information available online at <http://bio.biologists.org/lookup/doi/10.1242/bio.030726.supplemental>

References

Bross, T. G., Rogina, B. and Helfand, S. L. (2005). Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging Cell* **4**, 309–317.

Ceol, C. J., Houvras, Y., Jane-Valbuena, J., Bilodeau, S., Orlando, D. A., Battisti, V., Fritsch, L., Lin, W. M., Hollmann, T. J., Ferré, F. et al. (2011). The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset. *Nature* **471**, 513.

Clement, E., Lazar, I., Muller, C. and Nieto, L. (2017). Obesity and melanoma: could fat be fueling malignancy? *Pigment Cell Melanoma Res.* **30**, 294–306.

Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R. and Anderson, R. M. (2014). Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat. Commun.* **5**, 3557.

Erickson, K. L. (1984). Dietary fat influences on murine melanoma growth and lymphocyte-mediated cytotoxicity. *JNCI J. Natl. Cancer Inst.* **72**, 115–120.

Erickson, K. L., Gershwin, M. E., Canolty, N. L. and Eckels, D. D. (1979). The influence of dietary protein concentration and energy intake on mitogen response and tumor growth in melanoma-bearing mice. *J. Nutr.* **109**, 353–359.

Ershler, W. B., Berman, E. and Moore, A. L. (1986). Slower B16 melanoma growth but greater pulmonary colonization in calorie-restricted mice. *JNCI J. Natl. Cancer Inst.* **76**, 81–85.

Kaufman, C. K., Mosimann, C., Fan, Z. P., Yang, S., Thomas, A. J., Ablain, J., Tan, J. L., Fogley, R. D., van Rooijen, E., Hagedorn, E. J. et al. (2016). A zebrafish melanoma model reveals emergence of neural crest identity during melanoma initiation. *Science (New York, NY)* **351**, aad2197.

Klass, M. R. (1977). Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech. Ageing Dev.* **6**, 413–429.

Lian, C. G., Xu, Y., Ceol, C., Wu, F., Larson, A., Dresser, K., Xu, W., Tan, L., Hu, Y., Zhan, Q. et al. (2012). Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell* **150**, 1135–1146.

Lister, J. A., Capper, A., Zeng, Z., Mathers, M. E., Richardson, J., Paranthaman, K., Jackson, I. J. and Elizabeth Patton, E. (2014). A conditional zebrafish MITF mutation reveals MITF levels are critical for melanoma promotion vs. regression in vivo. *J. Invest. Dermatol.* **134**, 133–140.

Malvi, P., Chaube, B., Pandey, V., Vijayakumar, M. V., Boreddy, P. R., Mohammad, N., Singh, S. V. and Bhat, M. K. (2014). Obesity induced rapid melanoma progression is reversed by orlistat treatment and dietary intervention: role of adipokines. *Mol. Oncol.* **9**, 689–703.

Mattison, J. A., Colman, R. J., Beasley, T. M., Allison, D. B., Kemnitz, J. W., Roth, G. S., Ingram, D. K., Weindruch, R., de Cabo, R. and Anderson, R. M. (2017). Caloric restriction improves health and survival of rhesus monkeys. *Nat. Commun.* **8**, 14063.

Neiswender, J. V., Kortum, R. L., Bourque, C., Kasheta, M., Zon, L. I., Morrison, D. K. and Ceol, C. J. (2017). KIT suppresses BRAFV600E-mutant melanoma by attenuating oncogenic RAS/MAPK signaling. *Cancer Res.* **77**, 5820–5830.

Parichy, D. M., Elizondo, M. R., Mills, M. G., Gordon, T. N. and Engeszer, R. E. (2009). Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Dev. Dyn.* **238**, 2975–3015.

Patton, E. E., Widlund, H. R., Kutok, J. L., Kopani, K. R., Amatruda, J. F., Murphey, R. D., Berghmans, S., Mayhall, E. A., Traver, D., Fletcher, C. D. M. et al. (2005). BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr. Biol.* **15**, 249–254.

Piskounova, E., Agathocleous, M., Murphy, M. M., Hu, Z., Huddleston, S. E., Zhao, Z., Leitch, A. M., Johnson, T. M., Deberardinis, R. J. and Morrison, S. J. (2015). Oxidative stress inhibits distant metastasis by human melanoma cells. *Nature* **527**, 186–191.

Ravussin, E., Redman, L. M., Rochon, J., Das, S. K., Fontana, L., Kraus, W. E., Romashkan, S., Williamson, D. A., Meydani, S. N., Villareal, D. T. et al. (2015). A 2-year randomized controlled trial of human caloric restriction: feasibility and effects on predictors of health span and longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **70**, 1097–1104.

Rofe, A. M., Porter, S. J., Bais, R. and Conyers, R. A. (1985). The metabolic response of tumour-bearing mice to fasting. *Br. J. Cancer* **52**, 619–623.

Weindruch, R. and Walford, R. L. (1982). Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science (New York, NY)* **215**, 1415–1418.

White, R. M., Cech, J., Ratanasirintrawoot, S., Lin, C. Y., Rahl, P. B., Burke, C. J., Langdon, E., Tomlinson, M. L., Mosher, J., Kaufman, C. et al. (2011). DHODH modulates transcriptional elongation in the neural crest and melanoma. *Nature* **471**, 518–522.

Xia, S., Lin, R., Jin, L., Zhao, L., Kang, H.-B., Pan, Y., Liu, S., Qian, G., Qian, Z., Konstantakou, E. et al. (2017). Prevention of dietary-fat-fueled ketogenesis attenuates BRAF V600E tumor growth. *Cell Metab.* **25**, 358–373.