Journal of Student Research (2012) 1: 70-72

pac-1 enhances Ras-Raf-Erk Signaling in *C. elegans* Vulval Cell Fate Patterning

Brittany McMillan,[a] & Kimberly B. Monahan [a,b]

The Ras-Raf-Erk signaling pathway is essential for proper vulva development in *C.elegans* and proper response to stimuli in human cells. The importance of the conservation of these pathways shows how vulva development and cancer cells function like each other. The effect of Ras-Raf-Erk signaling in vulva development was examined by targeting *pac-1* with *RNAi* and then quantifying the number of vulva protrusions. The results from the experiment showed that the loss of *pac-1* inhibits ERK signaling in vulva development by decreasing the amount of vulva protrusions. This determined that normally *pac-1* increases ERK signaling in vulva development. The Ras-Raf-Erk pathway is activated in cancer and further understanding of the targets of this pathway will help us better understand how cancer develops.

Keywords: Ras-Raf-Erk pathway, vulva development, pac-1

Introduction

Ras and Ras-related small GTPases are essential signaling nodes that regulate how the cell works. Through numerous signaling networks, Ras controls many cellular processes, which include cell proliferation, gene expression, cell differentiation, cell growth, cell survival, cell apoptosis, intracellular vesicular trafficking, endocytosis/exocytosis, and regulation of the cytoskeleton and cell adhesion (reviewed in (Cox and Der, 2010 ; Santarpia et al., 2005)). Ras GTPases act as molecular switches through binding to GTP(Cox and Der) .When GTP-bound, Ras is turned "on" and when GDP- bound, Ras is turned "off." Activating mutations in Ras are found in approximately 30% of all human cancers (reviewed in (Adjei, 2001)). These mutations keep Ras in the GTP-bound state and drive cells to divide uncontrollably and cause cancer.

The *C. elegans* nematode worm has been an integral model organism for studying Ras signaling. The canonical Ras-Raf protein kinase cascade is highly conserved from worms to humans, and through genetic screens targeting the development of the vulva, novel signaling components and regulators of Ras signaling have been discovered. The hermaphrodite vulva connects the uterus to the outside environment and is necessary for egg laying and male copulation. A group of six epithelial cells called the vulval precursor cells (VPCs) along the ventral midline of the animal have the potential to assume vulval fate (Sternberg, 2005). Vulva development begins when the anchor cell secretes LIN-3/EGF, which binds to LET-23/EGFR found on the cell surface (Sundaram, 2006). Once EGFR is turned on it activates the Ras-Raf-Erk signaling cascade which is essential for proper vulval cell fate patterning and vulva function (Sundaram, 2006).

The major effector pathway of Ras is the Ras-Raf-Erk signaling pathway. This pathway is aberrantly regulated in many different cancers and developmental diseases. While the Ras-Raf-Erk pathway has been extensively studied, the downstream substrates of Erk (Ras-extracellular signal regulated kinase) have remained relatively elusive. Our lack of understanding of how and what Erk regulates has been an obstacle in the understanding of how this important pathway is involved in cancer and developmental diseases. Recently, Arur and colleagues identified novel Erk targets that affect *C.elegan* germline development (Arur et al., 2009). From their results a list of ERK targets was generated and we conducted a *RNAi* candidate screen of these targets in vulva cell fate patterning. As part of the screen using feeding *RNAi*, we identify *pac-1* as a potential target in Ras-Raf-Erk signaling in vulva development in *C.elegans*.

The target *pac-1* encodes a RhoGAP. RhoGAPs (Rho GTPase associating proteins) negatively regulate Rho GTPases, such as Rho, Rac and CDC-42 by hydrolyzing GTP to GDP. *pac-1* was shown to specifically inhibit Rho GTPase CDC-42 which caused defects in cellular polarization and cytoskeleton dynamics in developing *C.elegan* embryos (Anderson et *al*., 2008). Further research demonstrated that *pac-1* was found to be essential for proper recruitment of PAR proteins, PAR-6 and PAR-3 to cell contact surfaces and this proper recruitment was dependent on *pac-1* regulation of CDC-42 (Anderson et *al*., 2008). *pac-1* function in mammalian cells has not been studied; however it is speculated that *pac-1* would also regulate cellular polarization through PAR proteins. *pac-1* mediates how cell contact surfaces interact or regulate the polarization of cells within a developing embryo.

Experimental Procedures

Feeding RNAi

80 uL of feeding *RNAi* bacteria was plated on NGM agar containing 1 mM IPTG and 50 ug/ml carbenicillin as performed in (Fire et al., 1998; Kamath and Ahringer, 2003). *Gfp-1(RNAi)* was used as control, *pac-1(RNAi)* was used as experimental and *pop-1(RNAi)* was included as a positive control for *RNAi* efficacy because *pop-1 (RNAi)* causes embryonic lethality. The number of vulva protrusions was only scored if we observed 100% lethality on the *pop-1 (RNAi)* plates. On day 1, L4 (*let-60* (n1046gf)) animals were added to *RNAi* plates. After 48 hours, animals were transferred to new plates. At day 8,

a. Department of Natural Sciences, Fayetteville State University, NC
b. SPIRE Postdoctoral Fellowship Program, The University of North Carolina, NC

adult animals were scored for the number of vulva protrusions. All fRNAi experiments were performed at 16˚C.

Microscopy

Pictures of worms were taken at 40X with a SWIFT SM102 Stereoscope and images were acquired with the Motic Images Plus Version 2.0.

Statistical Analysis

The difference of number of vulva protrusions between each RNAi condition was analyzed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA) and compared using an unpaired, 2-tailed t-test. Error bars +/- standard error of the mean.

Results

Through genetic analysis targeting the development of the vulva, novel signaling components and regulators of Ras signaling have been discovered. Here, we use *RNAi* to dissect the role of *pac-1* in the Ras-Raf-Erk signaling pathway. Using this approach, we looked for a difference in the number of vulva protrusions with three potential outcomes: 1. enhancement of the number of vulva protrusions, 2. no change in number of vulva protrusions, 3. Suppression of the number of vulva protrusions. In an activating gain-of-function (gf) LET-60 background (*let-60* allele *n1046*; G13E mutation), we introduced *pac-1* (*RNAi*) or *gfp-1 (RNAi).* The LET-60 gain of function mutation was used because it is a mutation that is found in cancer and this would increase sensitivity in the Ras-Raf-Erk pathway. We found a significant decrease in the number of vulva protrusions when animals were grown on *pac-1* (*RNAi*) when compared to the *gfp-1* control *RNAi* (Figure 1A). To further support the results, pictures of adult animals with vulva protrusions on either *pac-1* (*RNAi*) or *gfp-1 (RNAi)* are shown in Figure 1B. These data suggest that *pac-1* has a positive signaling role in the Ras-Raf-Erk pathway. In *C.elegans* the effectiveness of RNAi is confirmed by using other assays such as deletion alleles. Therefore, while we cannot determine how effective our *RNAi* towards *pac-1* was in our assay, in future experiments we will use a deletion allele of *pac-1* in a LET-60 gain of function background to further confirm our RNAi results.

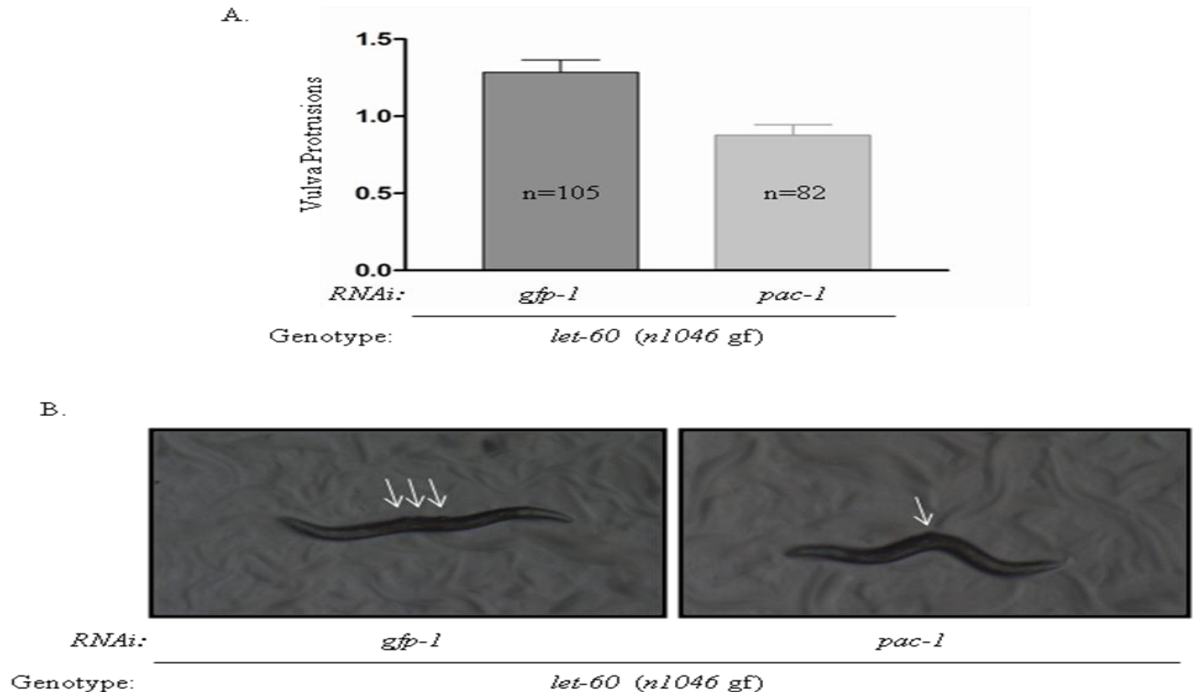

Figure 1: pac-1 decreases vulva protrusions: A. The graph shows the difference of number of vulva protrusions between the control RNAi gfp-1 and the target pac-1. A significant difference (p=0.0003) was seen between two RNAi conditions, showing a substantial decrease in vulva protrusions in pac-1 RNAi treated animals. B. Number of vulva protrusions from let-60 (n1046gf) C. elegans treated with gfp-1 or pac-1 (RNAi). gfp-1 RNAi treated animals developed more vulva protrusions; supporting that pac-1 RNAi decreases number of vulva protrusions.

Discussion

Pac-1 enhances Ras-Raf-Erk Signaling

The Ras-Raf-Erk signaling pathway is aberrantly regulated in many types of cancers. While the major components of the pathway have been established, the targets of Erk still remain fairly elusive. Here, using a list of novel Erk targets published in Arur *et al*, we tested the role of *pac-1* in Ras-Raf-Erk signaling. Through *RNAi* analysis we show that the loss of *pac-1* decreases the number of vulva protrusions in worms expressing an activated gain-of-function (gf) LET-60 allele. This suggests that normally *pac-1* enhances Ras-Raf-Erk signaling in vulval cell fate patterning. Downstream of the Ras-Raf-Erk pathway, *pac-1* regulates the activation of RhoGTPases and the dynamic interactions between cell contact surfaces and the polarity of cells. This would suggest the *pac-1* normally promotes proper structure formation of the vulva and how the vulva precursor cells interact with one another. Therefore loss of *pac-1* in a LET-60 gain of function background would cause a decrease in the number of vulva protrusions because they cannot properly form.

Pac-1 in Cancer Signaling

The results of the experiment impact cancer research by determining a target of the Ras-Raf-Erk pathway. This will help determine how to decrease the amount of cancerous cells being produced. My research shows that *pac-1* normally enhances the Ras-Raf-Erk pathway, so if we could inhibit *pac-1* expression this could decrease cancer cell growth.

Acknowledgements

I would like to thank my lab partner Doriesha Dalton and the BIO 430 class for helping me throughout the experimental and research process. This research was supported by the National Institutes of Health, Minority Opportunities in Research division of the National Institute of General Medical Sciences (NIGMS) grant #K12GM000678 and the Graduate School of Fayetteville State University STEM Faculty Mini-grant Program**.**

References

Adjei, A.A. (2001). Blocking oncogenic Ras signaling for cancer therapy. Journal of the National Cancer Institute *93*, 1062-1074.

Anderson, D. C., Gill, J.S., Cinalli, R.M., Nance, J. (2008) Polarization of the *C.elegans* Embryo by RhoGAP-mediated Exclusion of PAR-6 from Cell Contacts. Science 320 (5884), 1771-1774.

Arur, S., Ohmachi, M., Nayak, S., Hayes, M., Miranda, A., Hay, A., Golden, A., and Schedl, T. (2009). Multiple ERK substrates execute single biological processes in Caenorhabditis elegans germ-line development. Proceedings of the National Academy of Sciences of the United States of America *106*, 4776-4781.

Cox, A.D., and Der, C.J. (2010) Ras history: The saga continues. Small GTPases *1*, 2-27.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806-811.

Kamath, R.S., and Ahringer, J. (2003). Genome-wide RNAi screening in Caenorhabditis elegans. Methods *30*, 313-321.

Santarpia, L., Lippman, S.M., and El-Naggar, A.K. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. Expert opinion on therapeutic targets.

Sternberg, P.W. (2005). Vulval development. WormBook, 1-28.

Sundaram, M.V. (2006). RTK/Ras/MAPK signaling. WormBook, 1-19.