PAR-5 is a PARty hub in the germline
Multitask proteins in development and disease

David Aristizábal-Corrales,1,2,3 Simo Schwartz Jr1,2 and Julián Cerón3,*
1Drug Delivery and Targeting; CIBBIM-Nanomedicine; Vall d’Hebron Research Institute; Universidad Autónoma de Barcelona; Barcelona, Spain; 2Networking Research Center on Bioengineering; Biomaterials and Nanomedicine (CIBER-BBN); Barcelona, Spain; 3Department of Cancer and Human Molecular Genetics; Bellvitge Biomedical Research Institute (IDIBELL); L’Hospitalet de Llobregat; Barcelona, Spain

As our understanding of how molecular machineries work expands, an increasing number of proteins that appear as regulators of different processes have been identified. These proteins are hubs within and among functional networks. The 14-3-3 protein family is involved in multiple cellular pathways and therefore influences signaling in several disease processes, from neurobiological disorders to cancer. As a consequence, 14-3-3 proteins are currently being investigated as therapeutic targets. Moreover, 14-3-3 protein levels have been associated with resistance to chemotherapies. There are seven 14-3-3 genes in humans, while C. elegans only possesses two, namely par-5 and ftt-2. Among the C. elegans scientific community, par-5 is mainly recognized as one of the par genes that is essential for the asymmetric first cell division in the embryo. However, a recent study from our laboratory describes roles of par-5 in germ cell proliferation and in the cellular response to DNA damage induced by genotoxic agents. In this review, we explore the broad functionality of 14-3-3 proteins in C. elegans and comment on the potential use of worms for launching a drugs/modifiers discovery platform for the therapeutic regulation of 14-3-3 function in cancer.

Introduction

As genetic studies progress, the concept of “genetic pathways” tends to become obsolete and instead, sophisticated “genetic networks” arise as central controls of cellular and developmental processes. Within these networks, there are genes interconnecting nodes of a specific cellular or developmental process (party hubs) or genes that works as higher-level connectors of different functional modules (date hubs). While there is an ongoing active debate about the meaning of these terms, a recent study suggests that party and date hubs are not exclusive. 14-3-3 proteins form homo- and heterodimers that bind many phospho-Serine and phospho-Threonine-containing ligands to participate in the regulation of diverse functional networks. The interaction with 14-3-3 proteins is required to control stability, subcellular location, and activity of these phosphorylated ligands. Since there are seven 14-3-3 genes in humans (β, γ, ε, ζ, η, σ, and τ), and most of them are ubiquitously expressed, a wide range of influence in human physiology can be expected. In that regard, the existence of only two 14-3-3 genes in C. elegans (par-5 and ftt-2) helps to study hallmark functions of the family. In addition, par-5 is the only 14-3-3 gene expressed in the worm germline, while both ftt-2 and par-5 are expressed in the soma. Therefore, by characterizing the role of par-5 in the worm germline, we may be exploring the core functions of the protein family in germ cells through evolution. A search in Pubmed in July 2012 identified 4,000 publications related to 14-3-3 proteins, and one quarter of those entries also contained the term “disease,” which underscores the relevance of this protein family.
in biomedical research. However, there is still a limited understanding of the consequences of altered 14-3-3 function at the organism level.

**PAR-5: Polarity and Many Other Things**

Although the previous name of *par-5* was *fit-1* (14-three-three-1), the extensive research on the first embryonic division favored the use of *par-5*. The *C. elegans* first embryonic division is asymmetric and establishes the anterior-posterior axis that is essential for specifying the fates of daughter cells. Several genes known as *par* genes (from *partitioning defective*) have been found to be indispensable for this asymmetric cell division. The asymmetry of this division is not only due to the different size of daughter cells, but also to differential segregation of cell determinants. In 2002, Morton et al. showed that although PAR-5 is not asymmetrically distributed in the one-cell embryo, it is required for the asymmetric cortical localization of other PAR proteins and cell fate factors, such as MEX-5 and P-Granules, and is essential for the establishment of distinct posterior and anterior domains. In addition, during early embryonic stages, PAR-5 regulates the asymmetric nucleic enrichment of the transcription factor POP-1 upon WNT signaling, which is required for endoderm cell fate determination. In support of these essential functions in the embryo, *par-5* mutants display embryonic lethality.

Interestingly, beside the previously described functions of *par-5* in the embryo, our recent manuscript provided additional information regarding the role of *par-5* in the embryonic cell cycle. By measuring the cell cycle length at the first embryonic division, we found that *par-5*-defective embryos presented a shorter S-phase and a longer M-phase, suggesting that *par-5* is involved in the regulation of the embryonic cell cycle. At first sight, this cell cycle defect could be explained as a consequence of the impaired asymmetry, and therefore it should be observed after the suppression of other *par* genes. However, by analyzing videos from the Phenobank (www.worm.mpi-cbg.de), we found that such a cell cycle alteration seems to be unique among PAR family members. Furthermore, we showed that *par-5* is required for checkpoint-mediated delay of the first embryonic cell cycle in response to replicative stress. The checkpoint pathway contributes to asynchronous cell division occurring at the two-cell stage, but *par-5* suppression produces synchronous cell division of the two-cell embryo. Therefore, *par-5* has at least two separate roles in early embryonic development: one in asymmetric division and other in the regulation of cell cycle timing. Accordingly, PAR-5 could be a link between PAR family functions and embryonic cell cycle regulation as a mechanism for proper determination of cellular fates in the early embryo. Consequently, PAR-5 could be defined as a “party hub,” or a local coordinator in the process of the first embryonic division.

However, *par-5* has many other essential functions in the organism. Using co-immunoprecipitation and immunofluorescence experiments, *PAR-5* was found to interact with MAU-8, which is a protein required for G-protein signaling that regulates diverse physiological functions and behavior in *C. elegans*. Using Yeast Two Hybrid and co-immunoprecipitation assays, it was shown that PAR-5 and FTT-2 interact with LET-756, which is one of the two Fibroblast Growth Factors (FGFs) in *C. elegans* and is essential for development. PAR-5 and FTT-2 are binding partners of SIR-2.1 and are required for the life span extension conferred by high levels of SIR-2.1. These interactions are also functionally related to the stress response mediated by SIR-2.1 and DAF-16. Moreover, PAR-5 and FTT-2 also interact with DAF-16, and overexpression of either of the two *C. elegans* 14-3-3 proteins extends life span in a *daf-16*-dependent manner. However, the role of PAR-5 in aging is still controversial, since more recent studies underscore FTT-2 as the main 14-3-3 protein regulating lifespan, DAF-16 subcellular location, and expression of DAF-16 downstream targets. Finally, it has been recently reported that PAR-5 plays a role in apicobasal cell polarity in intestinal cells by regulating the polarized location of endosomes and F-actin. Moreover, we have added insight into the growing list of PAR-5 functions in *C. elegans* development and physiology by describing its role in germ cell proliferation and DNA damage response. In summary, higher or lower PAR-5 levels affect different biological processes or functional modules that are somehow related at the organism level. Future research will determine how these diverse PAR-5 functions are connected. Therefore, since PAR-5 regulates multiple functions by interacting with many proteins at distinct tissues, it may also be termed a “date hub.”

**Influence of PAR-5 in the Cell Cycle and DNA Damage Response: Levels, Levels, Levels**

As the list of PAR-5 functions expands, fine genetic approaches for dissecting out those functions are required. The power of biochemistry is diluted in multicellular organisms, where protein-protein interactions can vary between cell types. In this regard, biochemical studies may be noisy and too much of a reductionist approach. The amenability of *C. elegans* genetics to disrupt gene expression at different levels as well as at different lineages is an extraordinary tool that can be used to explore the diverse roles of multifunctional proteins. RNAi is an essential ingredient in any of these knockdown recipes. In our study, we worked with two different levels of *par-5* inactivation, including the use of a hypomorphic allele (point mutation, allele *is55*) that produces a reduction in PAR-5 levels, and an RNAi protocol that caused greater protein depletion. This approach allowed us to find that two different *par-5* expression levels are required for distinct *par-5* functions in the germ line (Table 1). Based on those results, it was clear that a mild decrease in PAR-5 levels is enough to affect the extrinsic DNA damage-induced checkpoint response, whereas a stronger depletion of the protein affects germ cell cycle progression and DNA stability. The correlation between the *par-5* levels and germ line defects was further confirmed by two observations: (1) the attenuated phenotypes observed when *par-5* RNAi was either diluted or administrated at later stages of development, and (2) the
stronger phenotypes observed when treating \textit{par-5} hypomorphic mutants with \textit{par-5} RNAi. Nevertheless, the functional dissection of a 14-3-3 protein could be assessed further in a multicellular organism such as \textit{C. elegans} by using RNAi approaches of different strengths through microinjection or by feeding with diluted dsRNA. Moreover, since there are strains that allow for lineage- or tissue-specific RNAi induction,\textsuperscript{16–18} researches have the opportunity to choose multiple options for exploring the function of a multitask protein such as \textit{PAR-5}.

To complete the range of \textit{par-5} levels that can be studied in \textit{C. elegans}, we have made a transgenic strain carrying a translational construct of \textit{PAR-5} in frame with GFP [\textit{par-5} promoter::GFP::\textit{par-5} ORF + \textit{par-5} 3’UTR] (Fig. 1A). This transgenic worm was generated by gene bombardment, and since the transgene is transmitted to 100% of the progeny, it may be integrated in the genome. However, we were not able to detect \textit{PAR-5}:GFP in the germline, although the GFP signal is very bright in somatic cells. This is most likely due to silencing of exogenous DNA in the germline. As a consequence, we did not mention the existence of this transgenic animal in our publication because it was irrelevant for studies of the germline. However, this strain can be useful for studying the effect of higher \textit{PAR-5} levels (assuming that the \textit{PAR-5} protein tagged with GFP is functional) in the chemoresistance of somatic cells or in aging. Moreover, this transgenic could be a valuable tool to study how 14-3-3 expression and subcellular localization are regulated by other pathways, physiological signals, or putative therapeutic small molecules (Fig. 1B).

The Use of Worms to Unravel the Roles of 14-3-3 Proteins in Cell Proliferation and Apoptosis

Since 14-3-3 proteins participate in many signaling networks, abnormal 14-3-3 functions and also deregulation of 14-3-3/par-5 partners interactions contribute to different types of diseases. 14-3-3 proteins self-assemble into homo- and heterodimers with other family members. These 14-3-3 dimers can interact with many other proteins (over 200) and thereby regulate their activity, and some of these binding partners are proteins with active roles in tumorigenesis, such as Raf proteins, FOXO1, and components of the TORC1 and β-catenin signaling pathways.\textsuperscript{20} Such is the promiscuity of 14-3-3s to interact with proteins that both pro-proliferative roles and tumor suppressor activities have been attributed to this family, depending on the cellular context.\textsuperscript{20} For the sake of simplicity, 14-3-3s have been divided in two groups according to their role in cancer: 14-3-3σ is a tumor suppressor, while the remaining 14-3-3 family members are oncogenes.\textsuperscript{21}

14-3-3σ, is expressed primarily in epithelial cells, which is in contrast to other family members.\textsuperscript{20} It is cataloged as a tumor suppressor because it positively regulates p53 and is also required for the G2/M checkpoint upon DNA damage.\textsuperscript{21} However the contribution of 14-3-3σ to tumorigenesis needs to be further explored. Other reports have associated 14-3-3σ expression with poor prognosis of diverse types of cancer, but this effect could be related to its role in chemoresistance. On the other hand, 14-3-3ζ overexpression has been detected in multiple cancers, and it has been suggested that this protein regulates signaling during cancer initiation and progression.\textsuperscript{21} In this sense, it will be interesting to investigate these dual roles of the 14-3-3 family in worms by studying: (1) the effect of higher or lower levels of \textit{PAR-5} and FTT-2 in cell proliferation during different \textit{C. elegans} developmental stages, and (2) the redundant or specific functions of \textit{PAR-5} and FTT-2 in somatic tissues where they are co-expressed.

Another mechanism by which 14-3-3 proteins influence cancer development is through apoptosis by interacting with BCL-2 family members.\textsuperscript{24,25} 14-3-3 proteins bind several effectors of apoptosis and inhibit their pro-apoptotic functions. As a consequence, 14-3-3 proteins have an anti-apoptotic effect that may contribute to tumorigenesis. In \textit{C. elegans}, physiological apoptosis can be studied either in the embryo or in the adult germline during oogenesis. We did not observe obvious alterations in apoptosis in the hypomorphic mutant (\textit{it55} allele), and our \textit{par-5} RNAi protocol severely impaired oocyte formation, which hampered the study of apoptosis. Therefore, a specific RNAi protocol needs to be optimized in order to study the role of \textit{par-5} in physiological apoptosis. Nevertheless, we observed nuclear fragmentation in proliferating germ cells of \textit{par-5} RNAi animals that resembles mitotic catastrophe, which is a process that leads to apoptosis. However, such fragmentation was triggered by premature mitotic entry, rather than by deregulation of pro-apoptotic proteins.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{PAR-5 protein expression} & \textbf{par-5(it55) mutant} & \textbf{par-5(RNAi)} \\
\hline
\textbf{In Unchallenged worms} & & \\
\hline
Germline proliferation defects & +/- & ++ \\
DNA damage in mitotic germ cells & - & + \\
DNA damage in meiotic germ cells & - & + \\
Meiotic progression defects & - & + \\
Sterility & - & + \\
\hline
\textbf{Upon exogenous DNA damage} & & \\
Checkpoint defects in germ cells & + & + \\
Checkpoint defects in embryo & + & + \\
Premature mitotic entry in germ cells & + & + \\
\hline
\end{tabular}
\caption{\textit{par-5} functions depend on \textit{PAR-5} levels}
\end{table}

Phenotypes observed in \textit{par-5(it55)} mutants vs. \textit{par-5} RNAi-treated animals. Phenotypes were examined in worms without any treatment (unchallenged worms), or worms treated with DNA damaging agents like Hydroxyurea, Ionizing Radiation and Camptothecin (exogenous DNA damage). According to their penetrance, phenotypes were classified as: strong (++), evident (+), mild (+/-) or absent (-).
indicates that this is a mechanism that seems to be conserved from worms to humans. Several studies have shown a correlation between high 14-3-3 levels and drug resistance. 14-3-3σ is one of the proteins that is upregulated in drug resistant cell lines, including breast cancer and pancreatic adenocarcinoma. Accordingly, overexpression of 14-3-3σ makes certain cell lines more resistant to chemotherapeutic drugs, such as cisplatin or gemcitabine. Conversely, 14-3-3σ depletion is correlated with increased sensitivity of colorectal cancer cells to doxorubicin-induced apoptosis. This effect on chemoresistance within the 14-3-3 family does not seem to be exclusive for 14-3-3σ. 14-3-3ζ was shown to mediate the resistance of lymphomas to anthracycline-based chemotherapies. Moreover, higher 14-3-3ζ expression is induced by tamoxifen, and this effect may lead to tamoxifen resistance in breast cancer. In addition, cancer treatment using doxorubicin and epirubicin has been shown to be less effective in tumors that overexpress 14-3-3ζ. Other 14-3-3 isoforms have also been shown to be involved in chemoresistance, either in cell lines or in patients. However, one recent study has shown that 14-3-3σ is downregulated in 5-fluorouracil (5-FU)-resistant breast cancer cells. This finding seems to be an exception, since 14-3-3 upregulation is correlated with resistance after most chemotherapeutic treatments.

The contribution of 14-3-3 proteins in the resistance to DNA-damaging drugs has been mainly associated with their function in suppressing cell death pathways, such as by regulating the pro-apoptotic protein BAD. However, this resistance may also be related to its role in regulating the cell cycle checkpoint after DNA damage. In support of this hypothesis, colorectal cancer cells fail to arrest at G2 upon DNA damage, but undergo mitotic catastrophe if 14-3-3σ is inactivated. This observation is very interesting, since 14-3-3 upregulation is correlated with resistance after most chemotherapeutic treatments.

Therefore, although it is evident that 14-3-3s have a profound impact in cancer progression, such impact is complex. Despite this complexity, 14-3-3 proteins still present high interest as therapeutic targets. Small molecule inhibitors that broadly affect 14-3-3 interactions or specifically inhibit interactions of 14-3-3 with certain binding partners could be of great interest to tackle cancer progression.

Chemoresistance as a Family Business

14-3-3 levels are also relevant to the resistance or sensitivity to chemotherapies, and interestingly, our recent study...
In summary, 14-3-3s play key roles in cancer-related processes, both in cancer progression and in the regulation of the sensitivity to chemotherapeutic agents. Crystal structures of all seven mammalian 14-3-3 proteins have been solved, and this information has facilitated the prediction of interactions with small molecules. However, the fact that 14-3-3s could interact with hundreds of proteins increases the probability of side effects for these potential drugs. Still, approaches for developing drugs that inhibit or modulate 14-3-3 functions are ongoing. One of the first attempts to inhibit 14-3-3 function led to the identification of a high affinity 14-3-3 agonist, the R18 peptide. R18 competes with natural client proteins for binding at the 14-3-3 amphipathic groove, and inhibits all 14-3-3 isoforms. However, more recently, the search has been focused on identifying non-peptidic inhibitors. For instance, Cotylecin A, which is a fungal metabolite, has been found to modify 14-3-3 interactions, and further derivatives of this drug could be synthesized in order to confer specificity for individual 14-3-3/target protein complexes. Another non-peptidic inhibitor, called BV02, has been found by virtual screening to sensitize leukemia cells in response to imatinib treatment. Chemical screenings have also identified a small 14-3-3 inhibitor molecule named FOBISIN (Fourteen-three-three Binding Small Molecule Inhibitor). Interestingly, inactivation of the 14-3-3ζ protein with this molecule is stabilized upon treatment of cells with X-rays, which may serve to develop radiation-triggered therapeutic agents for cancer treatment.

The relevance of 14-3-3s in controlling physiological, cellular, and molecular processes is unquestionable, and therefore understanding of 14-3-3 functions is of pivotal importance for treating diseases. However, the fact that humans possess seven 14-3-3 proteins that interact with more than 200 other proteins is a bottleneck in the understanding of their global functions. Many of these proteins, identified through proteomic approaches, are potential binding partners, but these interactions require a functional validation. It is now even clearer that core 14-3-3 functions are conserved through evolution, and therefore C. elegans is an extraordinary model to validate binding interactions assessing their capacity to phenocopy 14-3-3 inactivation or modify 14-3-3 functions (Fig. 1B). A drug that broadly modifies 14-3-3 interactions would be beneficial for certain diseases and chemotherapies, and the search of such a drug would begin in a model organism like C. elegans that possesses only two 14-3-3 proteins, which may represent the core functions of all family members (Fig. 1B). Finally, genetic engineering techniques based on the mos-1 transposon allow specific gene substitution in C. elegans. In that sense, a worm could be “humanized” for a specific 14-3-3 protein, for example by exchanging par-5 with 14-3-3ζ, in order to screen for drugs affecting only the 14-3-3 homodimer of interest. This replacement using different 14-3-3 genes could also allow for the identification of the individual contributions of 14-3-3 isoforms to the cell cycle and DNA damage response pathways, which in other words, would help to check the functional redundancy of family members.

Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

Acknowledgments We thank Montserrat Porta-de-la-Riva and Alberto Villanueva for the critical reading of this manuscript.

References

1. Han JD, Berriin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, et al. Evidence for dynamically organized modularity in the yeast protein-protein interaction network. Nature 2004; 430:88-93; PMID:15190252; http://dx.doi.org/10.1038/nature02555
2. Agarwala S, Deane CM, Porter MA, Jones NS. Revisiting date and party hubs: novel approaches to role assignment in protein interaction networks. PLoS Comput Biol 2010; 6:e1000817; PMID:20585543; http://dx.doi.org/10.1371/journal.pcbi.1000817
3. Wang W, Shades DC. Expression patterns and transcript processing of frt-1 and frt-2, two C. elegans 14-3-3 homologues. J Mol Biol 1997; 268:619-30; PMID:9171285; http://dx.doi.org/10.1006/jmbi.1997.1002
4. Suzuki A, Ohno S. The PAR-αPKC system: lessons in polarity. J Cell Sci 2006; 119:979-87; PMID:16525119; http://dx.doi.org/10.1242/jcs.02898
5. Morton DG, Shakes DC, Nugent S, Dichioso D, Wang W, Golden A, et al. The Caenorhabditis elegans par-5 gene encodes a 14-3-3 protein required for cellular asymmetry in the early embryo. Dev Biol 2002; 241:47-58; PMID:11784094; http://dx.doi.org/10.1006/dbio.2001.0489
6. Lo MC, Gay F, Odoni R, Shi Y, Lin R. Phosphorylation by the beta-catenin/MAPK complex promotes 14-3-3-mediated nuclear export of TCF/LEF-1 signal-responsive cells in C. elegans. Cell 2004; 117:95-106; PMID:15066285; http://dx.doi.org/10.1016/S0092-8674(04)00203-X
7. Aristizábal-Corrales D, Fontrodona L, Porta-de-la-Riva M, Guerra-Moreno A, Ceron J, Schwartz S Jr. The 14-3-3 gene par-5 is required for germ-line development and DNA damage response in Caenorhabditis elegans. J Cell Sci 2012; 125:1716-26; PMID:22328524; http://dx.doi.org/10.1242/jcs.098496
8. Brauchle M, Baumer K, Gönczy P. Differential activation of the DNA replication checkpoint contributes to asynchronous of cell division in C. elegans embryos. Curr Biol 2003; 13:819-27; PMID:12757829; http://dx.doi.org/10.1016/S0960-9822(03)00295-1
9. Lacoste C, Barthaux V, Ibotta C, Seagar M, Erard-Garcia M. MAU-8 is a Phosducin-like Protein required for G protein signaling in C. elegans. Dev Biol 2006; 294:181-91; PMID:16580661; http://dx.doi.org/10.1016/j.ydbio.2006.02.039
10. Popovic C, Berda Y, Conchonaud F, Harbis A, Birnbaum D, Roubin R. Direct and heterologous approaches to identify the LET-756/FGF targetrome. BMC Genomics 2006; 7:105; PMID:16679074; http://dx.doi.org/10.1186/1471-2164-7-105
11. Berdichevsky A, Viswanathan M, Horvitz HR. GuanTene L. C. elegans SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. Cell 2006; 125:1165-77; PMID:16777605; http://dx.doi.org/10.1016/j.cell.2006.03.026
12. Wang, Y, Oh SW, Deplancke B, Luo J, Wallbou AJ, Tissenbaum HA. C. elegans 14-3-3 proteins regulate life span and interact with SIR-2.1 and DAF-16/FOXO. Mech Ageing Dev 2006; 127:741-7; PMID:16803733; http://dx.doi.org/10.1016/j.mado.2006.05.005
13. Araiz C, Chateau MT, Galas S 14-3-3 regulates life span by both DAF-16-dependent and -independent mechanisms in Caenorhabditis elegans. Exp Gerontol 2008; 43:505-19; PMID:18423931; http://dx.doi.org/10.1016/j.exger.2008.03.003
14. Li J, Tewari M, Vidal M, Lee SS. The 14-3-3 protein FTT-2 regulates DAF-16 in Caenorhabditis elegans. Dev Biol 2007; 301:82-91; PMID:17098225; http://dx.doi.org/10.1016/j.ydbio.2007.06.015
15. Winter JE, Hoptner S, Korn K, Farning BO, Bradshaw CR, Marsico G, et al. Caenorhabditis elegans screen reveals role of PAR-5 in RAB-11-recycling endosome positioning and apical/cell polarity. Nat Cell Biol 2012; 14:666-76; PMID:22634595; http://dx.doi.org/10.1038/ncl.2012.63
16. Calixto A, Cheluri D, Topalidou I, Chen X, Chafile M. Enhanced neuronal RNAi in C. elegans using SID-1. Nat Methods 2010; 7:554-9; PMID:20512143; http://dx.doi.org/10.1038/nmeth.1463
17. Qudora H, Inoue M, Hikita T, Köppen M, Hardin JD, Amano M, et al. Establishment of a tissue-specific RNAi system in C. elegans. Gene 2004; 303:166-73; PMID:15768178; http://dx.doi.org/10.1016/j.gene.2007.06.020
18. Zhanag JJ, Hunter CP. Tissue specificity of Caenorhabditis elegans enhanced RNA interference mutants. Genetics 2011; 188:235-7; PMID:21385728; http://dx.doi.org/10.1534/genetics.111.127209
21. Neal CL, Yu D. 14-3-3 proteins as potential onco genes. Semin Cancer Biol 2006; 16:203-13; PMID:16725345; http://dx.doi.org/10.1016/j.semcancer.2006.03.004

22. Wilker EW, Grant RA, Artim SC, Yaffe MB. A structural basis for 14-3-3sigma functional specificity. J Biol Chem 2005; 280:18891-8; PMID:15731107; http://dx.doi.org/10.1074/jbc.M500982200

23. Li Z, Liu JY, Zhang JT. 14-3-3sigma, the double-stratifin (SIC1)22-2683(19991001)21:14:2952::AID-ELPS2952-3.0.CO;2-H

24. Gardino AK, Yaffe MB. 14-3-3 proteins as signaling integration points for cell cycle control and apoptosis. Semin Cell Dev Biol 2011; 22:688-95; PMID:21945644; http://dx.doi.org/10.1016/j.secmdb.2011.09.008

25. Porter GW, Khuri FR, Fu H. Dynamic 14-3-3/client protein interactions integrate survival and apoptotic pathways. Semin Cancer Biol 2006; 16:193-202; PMID:16697216; http://dx.doi.org/10.1016/j.semcancer.2006.03.003

26. Zhao J, Meyerkord CL, Du Y, Khuri FR, Fu H. 14-3-3 proteins as potential therapeutic targets. Semin Cell Dev Biol 2011; 22:705-12; PMID:21983031; http://dx.doi.org/10.1016/j.secmdb.2011.09.012

27. Liu Y, Liu H, Han B, Zhang JT. Identification of 14-3-3sigma as a contributor to drug resistance in human breast cancer cells using functional proteomics analysis. Cancer Res 2006; 66:3248-55; PMID:16540677; http://dx.doi.org/10.1158/0008-5472.CAN-05-3801

28. Sinha P, Hutter G, Kötgen E, Dietel M, Schadendorf D, Lage H. Increased expression of epidermal fatty acid binding protein, coflin, and 14-3-3-sigma (stratifin) detected by two-dimensional gel electrophoresis, mass spectrometry and microsequencing of drug-resistant human adenocarcinoma of the pancreas. Electrophoresis 1999; 20:2952-60; PMID:10546833; http://dx.doi.org/10.1002/(SICI)1522-2683(19991001)21:14:2952::AID-ELPS2952-3.0.CO;2-H

29. Neupane D, Korc M. 14-3-3sigma Modulates pancreatic cancer cell survival and invasiveness. Clin Cancer Res 2008; 14:7614-23; PMID:19047086; http://dx.doi.org/10.1158/1078-0432.CCR-08-1366

30. Chan TA, Hwang PM, Hermeking H, Kinzler KW, Vogelstein B. Cooperative effects of genes controlling the G(2)/M checkpoint. Genes Dev 2000; 14:1584-8; PMID:10887152

31. Frazer J, Chang EC, Komm B, Lin CY, Vega VB, Liu ET, et al. Expression preference preferentially regulated by tammoxifen in breast cancer cells and correlations with clinical outcome. Cancer Res 2006; 66:7334-40; PMID:16849584; http://dx.doi.org/10.1158/0008-5472.CAN-05-4269

32. Maxwell SA, Li Z, Jaye D, Ballard S, Ferrell J, Fu H. 14-3-3sigma mediates resistance of diffuse large B cell lymphoma to an anthracycline-based chemotherapeutic regimen. J Biol Chem 2009; 284:22379-89; PMID:19522224; http://dx.doi.org/10.1074/jbc.M109.022418

33. Li Y, Zou L, Li Q, Haibe-Kains B, Tian R, Li Y, et al. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. Nat Med 2010; 16:214-8; PMID:20098429; http://dx.doi.org/10.1038/nm.2390

34. Sinha P, Kohl S, Fischer J, Hutter G, Kern M, Kötgen E, et al. Identification of novel proteins associated with the development of chemoresistance in malignant melanoma using two-dimensional electrophoresis. Electrophoresis 2000; 21:3048-57; PMID:11003322; http://dx.doi.org/10.1002/1522-2683(20000801)21:14<3048::AID-ELPS3048+3.0.CO;2-W

35. Varzio A, Grochola LF, Bond EE, Levine AJ, N. The BAD protein integrates survival signals and apoptosis. Semin Cell Dev Biol 2011; 22:688-95; PMID:21945648; http://dx.doi.org/10.1016/j.semcancer.2010.09.006

36. Ottmann C, Weyand M, Sassa T, Inoue T, Kato N, Wintinghofer A, et al. A structural rationale for selective stabilization of anti-tumor interactions of 14-3-3 proteins by cortxin A. J Mol Biol 2009; 386:913-9; PMID:19244612; http://dx.doi.org/10.1016/j.jmb.2009.01.005

37. Mancini M, Corradi V, Petta S, Barbieri E, Manetti F, Botta M, et al. A new nonpeptide inhibitor of 14-3-3 induces apoptotic cell death in chronic myeloid leukemia sensitive or resistant to imatinib. J Pharmacol Exp Ther 2011; 336:596-604; PMID:21041536; http://dx.doi.org/10.1124/jpet.110.172536

38. Zhao J, Du Y, Horton JR, Upadhyay AK, Lou B, Bai Y, et al. Discovery and structural characterization of a small molecule 14-3-3-protein-protein interaction inhibitor. Proc Natl Acad Sci U S A 2011; 108:16212-6; PMID:21908710; http://dx.doi.org/10.1073/pnas.1100012108

39. Robert VJ, Bessereau JL. Genome engineering by transgene-instructed gene conversion in C. elegans. Methods Cell Biol 2011; 106:65-88; PMID:22118274; http://dx.doi.org/10.1016/B978-0-12-544172-8.00003-7