Biochemical and Biophysical Research Communications 427 (2012) 232–235

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mini Review

Targeting SMARCAL1 as a novel strategy for cancer therapy

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ARTICLE INFO

Article history:
Received 22 August 2012
Available online 17 September 2012

Keywords:
SMARCAL1
DNA repair
DNA replication
Cell cycle
Chemotherapy

ABSTRACT

SMARCAL1 is a SNF2 chromatin-remodeling protein with ATP-dependent annealing helicase activity. Recent studies have shown that SMARCAL1 is involved in DNA damage repair and cell cycle progression. Deficiency of SMARCAL1 enhances the anticancer activity of chemotherapy agents and reverses cancer cell resistance to these agents. Therefore, targeting SMARCAL1 is an attractive therapeutic approach for cancers with defects in DNA damage repair or cell cycle checkpoints. Here, we review advances in our understanding of the biochemical and cellular functions of SMARCAL1 made over the recent years and discuss the rationale for development of SMARCAL1 inhibitors as novel anticancer therapies.

1. Introduction

Defects in the pathways of DNA damage repair may result in persistent genetic lesions that can lead to carcinogenesis. On the other hand, the mechanism by which chemotherapy and radiotherapy induce cancer cell apoptosis and thus cell death begins with DNA damage. Cancer cells are more sensitive to DNA-damaging agents than normal cells because of their intrinsic deficiencies in DNA damage repair. However, cancer cells have alternative pathways of DNA damage repair that can be activated by DNA-damaging chemotherapy drugs; as these repair progresses, the cells develop resistance to these drugs. Therefore, inhibiting DNA damage repair molecules could result in cell death by reversing the cells' resistance to cancer-killing drugs, an example of synthetic lethality. Dozens of DNA damage repair inhibitors have already been tested in clinical trials. Here, we summarize the functions of SMARCAL1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1), an ATP-driven annealing helicase member of the SNF2 family, in DNA damage repair and cell cycle progression and discuss the rationale for targeting SMARCAL1 as a strategy for cancer therapy.

2. The *SMARCAL1* gene

Structurally, SMARCAL1, also known as HepA-related protein (HARP), is highly similar to the *Escherichia coli* RNA polymerase binding protein HepA [1–3]. It is a SNF2 family member of ATP-dependent chromatin-remodeling enzymes. The SNF2 molecules act in gene transcription, DNA damage repair, DNA recombination, DNA methylation, and cell cycle regulation. SMARCAL1 contains a region of 28 amino acids at the *N* terminus; two tandem HARP domains, both of which contain ~60 residues (residues 239–307 and residues 331–400); and a SWI/SNF helicase domain [4]. SWI/SNF proteins belong to the SNF2 subfamily whose members contain seven characterized motifs (I, Ia, II, III, IV, V, and VI) [2]. SMARCAL1 contains the conserved helicase motifs that are highly similar to those other of SNF2 family members but lacks the motif III to motif IV regions [3]. The tandem HARP domains are crucial for its annealing helicase activity [5].

The *SMARCAL1* gene is ubiquitously expressed in various human and mouse tissues. Mutations in SMARCAL1 cause an autosomal recessive genetic disease, Schimke immuno-osseous dysplasia (SIOD), which is characterized by T-cell immunodeficiency and growth retardation due to skeletal dysplasia [6]. Patients with SIOD show defects in T-cell proliferation in response to mitogens such as interleukin-2, phytohemagglutinin, and concanavalin A. SIOD-associated anemia does not respond to erythropoietin, and SIOD-associated bone marrow failure does not respond to stem cell factors. The growth retardation does not improve with growth hormone supplementation. Those findings imply that SMARCAL1 is a crucial regulator of cell proliferation, cell differentiation, cell survival, or cell cycle regulation.

Unlike other members of the SNF2 family such as Rad54, ACF, and Brg1, which have DNA-unwinding activities, SMARCAL1

Abbreviations: ATM, ataxia telangiectasia-mutated; ATR, Rad3 related kinase; ATRIP, ATR interacting protein; chk2, checkpoint kinase 2; DSB, dsDNA break; HARP, HepA-related protein; RPA, replication protein A; SIOD, Schimke immuno-osseous dysplasia; SMARCAL1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily a-like 1; ssDNA, single-stranded DNA.

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http://dx.doi.org/10.1016/j.bbrc.2012.09.060

catalyzes the rewinding of single-stranded DNA (ssDNA) bubbles coated with replication protein A (RPA) [7]. The ATPase activity of SMARCAL1 is activated by the unique DNA structures of double-stranded DNA (dsDNA)–ssDNA junctions [8,9], including model replication forks [7]. The biological importance of SMARCAL1 in humans is revealed by its role in SIOD.

3. SNF2 ATP-dependent chromatin-remodeling complexes and cancer

The SNF2 complexes are present in all eukaryotes and are highly evolutionarily conserved. The purified yeast SWI/SNF complex, which has ATP-dependent activity, is a multimeric chromatin-remodeling complex important for gene regulation [7]. Among the subunits of the ATP-dependent chromatin-remodeling complexes, SWI/SNF-related ATPase provides its *N*- and *C*-terminal domains to interact with other proteins in the complex [10]. The ATPase derives specificity for chromatin domains through these interactions. Using the energy of ATP hydrolysis, the SWI/SNF ATPase alters the structure of DNA nucleosomes to remodel chromatin and open the DNA, and then it advances gene transcription and aids in DNA recombination, replication, and repair. It also regulates transcription of a series of interrelated genes involved in cell proliferation and differentiation [11].

Loss of the ATPase-dependent chromatin-remodeling complexes correlates with tumorigenesis. BRM (Brahma) and BRG1 (BRM-related gene 1) are two SWI/SNF ATPase-dependent chromatin-remodeling molecules. Four of 12 (33%) human non–small-cell lung cancer cell lines were found to have concomitant downregulation of BRG1 and BRM. Loss of both BRG1 and BRM, which depresses SWI/SNF complex activity, is more common than loss of either alone [12]. SMARCB1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1) is a core member of the SWI/SNF chromatin-remodeling complex. SMARCB1-knockout mice are prone to cancer, with a median onset of 11 weeks. The tumors are closely similar to human rhabdoid tumors with classic rhabdoid morphology [13].

The ATPase activity of the SWI/SNF members is stimulated by dsDNA breaks. Loss of this activity results in defects in DNA damage repair. In contrast, the ATPase activity of SMARCAL1 is activated by stem loop and forked DNA structures, more by ssDNA to dsDNA transitions with a recessed 3′-hydroxyl group than by ssDNA or dsDNA breaks *in vitro* [7].

4. SMARCAL1 in DNA damage repair and replication

Most SNF2 family members contain DEAD-box helicase-like domains that are activated in response to DNA damage and are involved in the progression of DNA repair and replication. For example, INO80 shows genetic interactions with the DNA repair pathway for dsDNA break (DSB) repair in yeast [14]. It is involved in replication forks for proper restart of DNA replication and repair of DSBs [15–17].

In an assay of microirradiated human cell lines A172, HeLa, IMR90, and MCF7, SMARCAL1 localizes to the sites of laser-induced DNA damage, implying that SMARCAL1 acts in DSB repair. And SMARCAL1 is recruited to DNA damage sites after Mre11 (meiotic recombination 11) [18], an initial component of homologous recombination (HR) and a key early response protein for processing the DNA ends of broken chromosomes for repair [19].

In cells treated with a DNA-damaging agent orreplication inhibitor, SMARCAL1 co-localizes with γ-H2AX, a marker of DSB, suggesting that SMARCAL1 could be recruited into sites of DNA damage [20]. SMARCAL1 is recruited to the damage foci in cells treated with hydroxyurea, which inhibits DNA synthesis and DNA repair in damaged cells [20,21]. SMARCAL1 also is recruited to the foci generated in cells treated with camptothecin, a topoisomerase I inhibitor that interferes with DNA damage repair and replication. Postow et al. found that SMARCAL1 is phosphorylated in a caffeine-sensitive manner that is involved in stabilizing the stalled forks. Loss of SMARCAL1 enhances RAD51 association with the stalled replication forks [22].

In SMARCAL1-deleted cells, γ-H2AX foci accumulate and both ataxia telangiectasia-mutated (ATM) and checkpoint kinase 2 (chk2) are activated. Levels of RPA-bound ssDNA are higher during S phase than in wild-type cells [21,23]. Bétous et al. verified recently that absence of SMARCAL1 leads to MUS8-dependent DSB formation and activation of an alternate repair mechanism [24]. These findings suggest that deficiency of SMARCAL1 increases DSBs and thus that SMARCAL1 is involved in the processes of DNA damage repair and replication [25].

Ciccia et al. found that SMARCAL1-depleted cells exhibit a delay in bromodeoxyuridine incorporation after treatment with the replication inhibitor thymidine [20]. Using SMARCAL1-deficient cells, several groups confirmed that SMARCAL1 is required for resistance to DNA-damaging agents such as camptothecin, mitomycin C, aphidicolin, and hydroxyurea, indicating a possible cell cycle–specific role for SMARCAL1 [4,20,21]. Re-introduction of wild-type SMARCAL1 to fibroblasts from patients with SIOD could rescue the elevated levels of DNA damage and increase resistance to DNA-damaging agents [26].

RPA binds to ssDNA, which recruits ATM, Rad3 related kinase (ATR), and the ATR interacting protein (ATRIP) and activates ATR to start the DNA damage checkpoint and repair processes [27,28]. Thus RPA is involved in DNA replication and DNA damage repair. Several groups have found that SMARCAL1 interacts with RPA2 [20]. Yuan et al. confirmed that SMARCAL1 interacts strongly with RPA1 [4]. These findings indicate that SMARCAL1 may combine more than one site with RPA complex. Instead of converting dsDNA into RPA-bound unwound DNA, SMARCAL1 functionally binds to forked DNA *in vitro* and rewinds the regions of RPA-bound ssDNA in a plasmid context [7]. The interaction between SMARCAL1 and the RPA complex suggests that SMARCAL1 functions during these processes or plays an important role in both DNA replication and repair (Fig. 1A).

5. SMARCAL1 and the cell cycle

Several lines of evidence suggest that SMARCAL1 is involved in regulation of the cell cycle. E2Fs are important transcription factors regulating the cell cycle through activation or suppression of their target genes, such as cyclins and cyclin-dependent kinases. We verified that E2F6 could bind to the human *SMARCAL1* gene promoter and inhibit its expression in 293T cells.

Ciccia et al. and Bansbach et al. showed that SMARCAL1 recruitment to the sites of ionizing radiation- or laser-induced DNA damage occurs only in S/G_2-phase cells, indicating that SMARCAL1 may be recruited to sites of damage only in the S and G_2 phases of the cell cycle [20,21]. This is consistent with the presumption that SMARCAL1 recognizes ssDNA generated during resection, a process that is restricted to S and G_2 [29]. Localization of SMARCAL1 to the sites of DNA damage required *C*-terminal binding protein-interacting protein (CtIP), which promotes DNA resection to generate ssDNA ends for HR repair of DSB only during the S and G_2 phases of the cell cycle [20,29,30]. This indicates that SMARCAL1 recognizes ssDNA generated by damaged cells during the S/G_2 phases and may play a role in HR DNA repair.

SMARCAL1-depleted cells show a defect in progress from S phase into mitosis. The arrest between S-phase and mitosis occurred in 75% fewer nocodazole-treated cells than control cells.

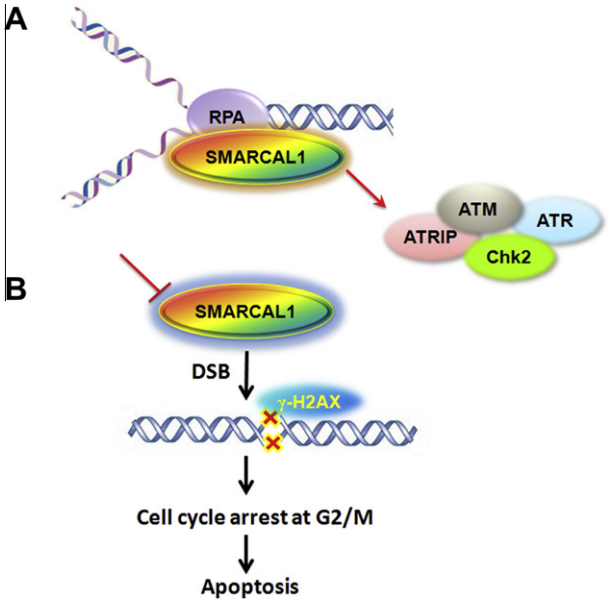

Fig. 1. Schematic diagram of SMARCAL1's role in DNA repair and cell cycle progression. (A) Fork of dsDNA break of RPA recruits SMARCAL1 and activates DNA-repair molecules. (B) Suppression of SMARCAL1 results in dsDNA break and leads to cell cycle arrest at G2/M phase and cell apoptosis.

In cells exposed to irradiation or camptothecin, this delay is exacerbated, suggesting that SMARCAL1-depleted cells are sensitive to DNA-damaging agents that induce DSBs during S phase. Meanwhile, there is a slight but reproducible increase (3%) in the G_2 population in the treated cells compared to the control cells [20]. This was confirmed by the results of Yuan et al. showing that the percentage of cells in G_2/M phase was increased while the percentages in G_1 and S phases were decreased in si*SMARCAL1*-treated human osteosarcoma U2OS cells. These data indicate that SMARCAL1 depletion results in cell cycle arrest in the G_2/M phase, which may induce cell apoptosis [4] (Fig. 1B). Furthermore, the fibroblasts reconstituted with SMARCAL1 exhibited faster cell cycle progression after S-phase arrest. In contrast, the cell cycle progression of fibroblasts from SIOD patients is much slower than that of wild-type fibroblasts [20]. This may result from defects in the transition from S phase to G_2 phase. These data suggest that SMARCAL1' involvement in DNA damage repair is related to cell cycle progression. Targeting SMARCAL1 may result in DNA repair defects, inhibiting cell cycle progression and inducing apoptosis.

In contrast to the findings just described, we demonstrated the involvement of SMARCAL1 in cell cycle regulation in zebrafish. In these fish, knockdown of SMARCAL1 suppressed expression of cyclinA2, which promotes the transition from G_1 to S phase [21]; increased expression of p21, an inhibitor of the transition from G_1 to S [23]; and caused cell cycle arrest at the G_0/G_1 phase. Furthermore, SMARCAL1 deficiency induced p53-independent cell apoptosis, suggesting that SMARCAL1 is required for the G_1/S phase transition in zebrafish [31].

6. Future directions

The annealing helicase activity of SMARCAL1 depends on ATP hydrolysis. The defects in ATPase and annealing helicase activity correlate with severity of SIOD. Therefore, ATPase activity is indispensable for SMARCAL1 functions. Cells in which SMARCAL1 has been mutated or knocked down have been shown to accumulate more DSBs than normal cells and to have greater sensitivity to chemotherapy. Furthermore, overexpression of SMARCAL1 protects cells against camptothecin-induced cell death, indicating that inhibition of SMARCAL1 may result in synthetic lethality in tumors with defects in DNA repair and induce hypersensitivity of cancer cells to DNA-damaging agents or DNA-replication inhibitors.

ATPase is an attractive drug target. Several ATPase inhibitors have already been developed and are widely used in the clinical setting [32]. Given SMARCAL1's ATPase activity and its role in DNA damage repair, and that SIOD patients are not prone to carcinogenesis, SMARCAL1 is an ideal drug target for cancer therapy. High-throughput screening of SMARCAL1 inhibitors is feasible, as SMARCAL1 can be expressed as a biologically active recombinant protein and its ATPase activity assay can be easily automated [33]. SMARCAL1 high-resolution crystal structures could provide a means for *in silico* screening and lead optimization. Inhibitors of SMARCAL1 may enhance the cytotoxicity of camptothecin and other DNA damage-inducing agents in cell culture, thereby providing a cellular platform for compound selection. The ultimate goal is to develop new drugs that would selectively increase the cytotoxicity of and reverse the resistance to DNA-damaging agents and replication inhibitors in tumors with defects in DNA repair and cell cycle checkpoint.

Conflicts of interest

The authors report no conflicts of interest.

Acknowledgments

Financial assistance was provided by grants from the National Natural Science Foundation of China (81173141) and 973 Project of National Key Basic Research of China (2007CB947100). We thank KL Hale for critical reading of this manuscript.

References

[1] J.A. Eisen, K.S. Sweder, P.C. Hanawalt, Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions, Nucleic Acids Res. 23 (1995) 2715–2723.
[2] M.A. Coleman, J.A. Eisen, H.W. Mohrenweiser, Cloning and characterization of HARP/SMARCAL1: a prokaryotic HepA-related SNF2 helicase protein from human and mouse, Genomics 65 (2000) 274–282.
[3] A. Flaus, D.M. Martin, G.J. Barton, T. Owen-Hughes, Identification of multiple distinct Snf2 subfamilies with conserved structural motifs, Nucleic Acids Res. 34 (2006) 2887–2905.
[4] J. Yuan, G. Ghosal, J. Chen, The annealing helicase HARP protects stalled replication forks, Genes Dev. 23 (2009) 2394–2399.
[5] G. Ghosal, J. Yuan, J. Chen, The HARP domain dictates the annealing helicase activity of HARP/SMARCAL1, EMBO Rep. 12 (2011) 574–580.
[6] T. Lucke, D. Franke, J.M. Clewing, C.F. Boerkoel, J.H. Ehrich, A.M. Das, M. Zivicnjak, Schimke versus non-Schimke chronic kidney disease: an anthropometric approach, Pediatrics 118 (2006) e400–e407.
[7] T. Yusufzai, J.T. Kadonaga, HARP is an ATP-driven annealing helicase, Science 322 (2008) 748–750.
[8] J.W. Hockensmith, A.F. Wahl, S. Kowalski, R.A. Bambara, Purification of a calf thymus DNA-dependent adenosinetriphosphatase that prefers a primer-template junction effector, Biochemistry 25 (1986) 7812–7821.
[9] R. Muthuswami, P.A. Truman, L.D. Mesner, J.W. Hockensmith, A eukaryotic SWI2/SNF2 domain, an exquisite detector of double-stranded to single-stranded DNA transition elements, J. Biol. Chem. 275 (2000) 7648–7655.
[10] K. Havas, I. Whitehouse, T. Owen-Hughes, ATP-dependent chromatin remodeling activities, Cell Mol. Life Sci. 58 (2001) 673–682.
[11] C.F. Boerkoel, H. Takashima, J. John, J. Yan, P. Stankiewicz, L. Rosenbarker, J.L. Andre, R. Bogdanovic, A. Burguet, S. Cockfield, I. Cordeiro, S. Frund, F. Illies, M. Joseph, I. Kaitila, G. Lama, C. Loirat, D.R. McLeod, D.V. Milford, E.M. Petty, F. Rodrigo, J.M. Saraiva, B. Schmidt, G.C. Smith, J. Spranger, A. Stein, H. Thiele, J. Tizard, R. Weksberg, J.R. Lupski, D.W. Stockton, Mutant chromatin remodeling protein SMARCAL1 causes Schimke immuno-osseous dysplasia, Nat. Genet. 30 (2002) 215–220.
[12] D.N. Reisman, J. Sciarrotta, W. Wang, W.K. Funkhouser, B.E. Weissman, Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis, Cancer Res. 63 (2003) 560–566.
[13] C.W. Roberts, J.A. Biegel, The role of SMARCB1/INI1 in development of rhabdoid tumor, Cancer Biol. Ther. 8 (2009) 412–416.

[14] A.J. Morrison, J. Highland, N.J. Krogan, A. Arbel-Eden, J.F. Greenblatt, J.E. Haber, X. Shen, INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair, Cell 119 (2004) 767–775.
[15] H. Durr, A. Flaus, T. Owen-Hughes, K.P. Hopfner, Snf2 family ATPases and DExx box helicases: differences and unifying concepts from high-resolution crystal structures, Nucleic Acids Res. 34 (2006) 4160–4167.
[16] M. Papamichos-Chronakis, C.L. Peterson, The Ino80 chromatin-remodeling enzyme regulates replisome function and stability, Nat. Struct. Mol. Biol. 15 (2008) 338–345.
[17] J.A. Vincent, T.J. Kwong, T. Tsukiyama, ATP-dependent chromatin remodeling shapes the DNA replication landscape, Nat. Struct. Mol. Biol. 15 (2008) 477–484.
[18] T. Yusufzai, X. Kong, K. Yokomori, J.T. Kadonaga, The annealing helicase HARP is recruited to DNA repair sites via an interaction with RPA, Genes Dev. 23 (2009) 2400–2404.
[19] J.S. Kim, T.B. Krasieva, H. Kurumizaka, D.J. Chen, A.M. Taylor, K. Yokomori, Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells, J. Cell Biol. 170 (2005) 341–347.
[20] A. Ciccia, A.L. Bredemeyer, M.E. Sowa, M.E. Terret, P.V. Jallepalli, J.W. Harper, S.J. Elledge, The SIOD disorder protein SMARCAL1 is an RPA-interacting protein involved in replication fork restart, Genes Dev. 23 (2009) 2415–2425.
[21] C.E. Bansbach, R. Betous, C.A. Lovejoy, G.G. Glick, D. Cortez, The annealing helicase SMARCAL1 maintains genome integrity at stalled replication forks, Genes Dev. 23 (2009) 2405–2414.
[22] L. Postow, E.M. Woo, B.T. Chait, H. Funabiki, Identification of SMARCAL1 as a component of the DNA damage response, J. Biol. Chem. 284 (2009) 35951–35961.
[23] A. Vidal, A. Koff, Cell-cycle inhibitors: three families united by a common cause, Gene 247 (2000) 1–15.
[24] R. Betous, A.C. Mason, R.P. Rambo, C.E. Bansbach, A. Badu-Nkansah, B.M. Sirbu, B.F. Eichman, D. Cortez, SMARCAL1 catalyzes fork regression and Holliday junction migration to maintain genome stability during DNA replication, Genes Dev. 26 (2012) 151–162.
[25] R. Driscoll, K.A. Cimprich, HARPing on about the DNA damage response during replication, Genes Dev. 23 (2009) 2359–2365.
[26] C.E. Bansbach, C.F. Boerkoel, D. Cortez, SMARCAL1 and replication stress: an explanation for SIOD?, Nucleus 1 (2010) 245–248
[27] L. Zou, S.J. Elledge, Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes, Science 300 (2003) 1542–1548.
[28] K.A. Cimprich, D. Cortez, ATR: an essential regulator of genome integrity, Nat. Rev. Mol. Cell Biol. 9 (2008) 616–627.
[29] A.A. Sartori, C. Lukas, J. Coates, M. Mistrik, S. Fu, J. Bartek, R. Baer, J. Lukas, S.P. Jackson, Human CtIP promotes DNA end resection, Nature 450 (2007) 509–514.
[30] L. Chen, C.J. Nievera, A.Y. Lee, X. Wu, Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair, J. Biol. Chem. 283 (2008) 7713–7720.
[31] C. Huang, S. Gu, P. Yu, F. Yu, C. Feng, N. Gao, J. Du, Deficiency of smarcal1 causes cell cycle arrest and developmental abnormalities in zebrafish, Dev. Biol. 339 (2010) 89–100.
[32] P. Chene, ATPases as drug targets: learning from their structure, Nat. Rev. Drug Discovery 1 (2002) 665–673.
[33] L.I. Elizondo, K.S. Cho, W. Zhang, J. Yan, C. Huang, Y. Huang, K. Choi, E.A. Sloan, K. Deguchi, S. Lou, A. Baradaran-Heravi, H. Takashima, T. Lucke, F.A. Quiocho, C.F. Boerkoel, Schimke immuno-osseous dysplasia: SMARCAL1 loss-of-function and phenotypic correlation, J. Med. Genet. 46 (2009) 49–59.