Mutation Research 791 (2016) 1–9

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

A pivotal role of the jasmonic acid signal pathway in mediating radiation-induced bystander effects in *Arabidopsis thaliana*

Ting Wang[a,b,1], Wei Xu[a,b,1], Chenguang Deng[a,b,1], Shaoxin Xu[a,b], Fanghua Li[a,b], Yuejin Wu[a,b], Lijun Wu[a,b], Po Bian[a,b,*]

[a] Key Laboratory of Ion Beam Bioengineering, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, PR China
[b] Key Laboratory of Environmental Toxicology and Pollution Control Technology of Anhui Province, Hefei 230031, PR China

ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 3 June 2016
Accepted 28 July 2016
Available online 30 July 2016

Keywords:
Radiation-induced bystander effect
Arabidopsis thaliana
Jasmonic acid
JA signal pathway
DNA repair

ABSTRACT

Although radiation-induced bystander effects (RIBE) in *Arabidopsis thaliana* have been well demonstrated *in vivo*, little is known about their underlying mechanisms, particularly with regard to the participating signaling molecules and signaling pathways. In higher plants, jasmonic acid (JA) and its bioactive derivatives are well accepted as systemic signal transducers that are produced in response to various environmental stresses. It is therefore speculated that the JA signal pathway might play a potential role in mediating radiation-induced bystander signaling of root-to-shoot. In the present study, pretreatment of seedlings with Salicylhydroxamic acid, an inhibitor of lipoxigenase (LOX) in JA biosynthesis, significantly suppressed RIBE-mediated expression of the *AtRAD54* gene. After root irradiation, the aerial parts of *A. thaliana* mutants deficient in JA biosynthesis (*aos*) and signaling cascades (*jar1-1*) showed suppressed induction of the *AtRAD54* and *AtRAD51* genes and *TSI* and *180-bp* repeats, which have been extensively used as endpoints of bystander genetic and epigenetic effects in plants. These results suggest an involvement of the JA signal pathway in the RIBE of plants. Using the root micro-grafting technique, the JA signal pathway was shown to participate in both the generation of bystander signals in irradiated root cells and radiation responses in the bystander aerial parts of plants. The over-accumulation of endogenous JA in mutant *fatty acid oxygenation up-regulated 2* (*fou2*), in which mutation of the Two Pore Channel 1 (*TPC1*) gene up-regulates expression of the *LOX* and *allene oxide synthase* (*AOS*) genes, inhibited RIBE-mediated expression of the *AtRAD54* gene, but up-regulated expression of the *AtKU70* and *AtLIG4* genes in the non-homologous end joining (NHEJ) pathway. Considering that NHEJ is employed by plants with increased DNA damage, the switch from HR to NHEJ suggests that over-accumulation of endogenous JA might enhance the radiosensitivity of plants in terms of RIBE.

1. Introduction

Radiation-induced bystander effects (RIBE) represent a paradigm shift in our understanding of the radiobiological effects of ionizing radiation, in which radiation responses in non-hit cells can be induced by the signals from nearby hit cells [1]. Bystander effects have been well demonstrated using a wide range of biological endpoints in single-cell culture models [2–5], multi-cellular tissue models [6–11], and whole animals [12–19]. Recently, our team has started to investigate RIBE using the model plant *Arabidopsis thaliana*. Following microbeam-localized irradiation of naked seed embryos and low-energy ion irradiation of intact seeds, some post-embryonic developmental phenotypes were significantly changed, differentiating from the non-irradiated shoot apical meristem cells and root apical meristem cells, respectively [20,21]. The enhanced level of DNA strand breaks, up-regulated expressions of the DNA damage repair gene *AtRAD54*, and increased induction of DNA homologous recombination (HR) have been observed in the non-irradiated aerial parts of plants after local irradiation of roots with alpha particles and dormant seeds with low-energy argon ions, indicating the occurrence of bystander mutagenic effects

Abbreviations: HR, homologous recombination; NHEJ, non-homologous end-Joining; TGS, transcriptional gene silencing; JA, jasmonic acid; MeJA, methyl jasmonate; TSI, transcriptionally silent information; GUS, uidA (β-glucuronidase) gene; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SHAM, salicylhydroxamic acid; ROS, reactive oxygen species; *JAR1*, *JASMONATE RESISTANT 1*; LOX, lipoxigenase; AOS, allene oxide synthase; AOC, allene oxide cyclase.

* Corresponding author at: P.O. Box 1138, Hefei, Anhui 230031, PR China.
E-mail address: bianpo@ipp.ac.cn (P. Bian).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.mrfmmm.2016.07.002
0027-5107/

in plants [22,23]. Bystander effects in plants were also found to mediate changes at the epigenetic level, such as the level and pattern of DNA methylation, and the alleviation of transcriptional gene silencing (TGS) [24]. Interestingly, in contrast to normal experimental conditions, the bystander effects in plants have some distinct manifestations under modeled microgravity conditions, due to modulation of the generation and/or transportation of bystander signaling molecules by microgravity in irradiated root cells [25]. In order to investigate the time course of root-to-shoot bystander signaling, young seedlings of *A. thaliana* were treated with a combination of root micro-grafting and root irradiation, whereby the root-to-shoot bystander signaling could easily be stopped or started at specific time points [26]. On the basis of this technique, we further demonstrated temporal and spatial features of bystander signaling and the synergistic effects of multiple bystander signals in *A. thaliana* [26,27].

However, the molecular mechanisms underlying RIBE in plants remain to be determined, particularly with regard to the signaling molecules and signaling cascades. It has been reported that in cell culture models, some signal factors are released from hit cells and induce radiation responses in bystander cells through diffusion in medium and/or cellular gap-junctions [1,2]. Several signaling cascades, including MAPK, NF-kB/cox-2, NO^-, and inflammation-related pathways, are thought to mediate RIBE transduction [1,28–30]. Although RIBE have also been demonstrated in whole animal models, there is little information as to the nature of long-distance bystander communication [15,17,31]. In marked contrast to DNA damage in irradiated tissues, which is mainly caused by direct energy deposition on DNA strands and/or oxidative damage by free radicals produced by lysis of water [32], the DNA damage and resultant DNA repairs in bystander tissues is generally attributable to enhanced oxidative stress triggered by the incoming bystander signals [33]. Plant hormones such as jasmonic acid (JA) and salicylic acid have been reported to increase the induction of HR and activation of TGS loci [34,35]. Interestingly, the distribution and level of the plant hormone auxin in bystander tissues can be changed by microbeam-localized irradiation of naked seeds of *A. thaliana* [20]. Accordingly, it is proposed that plant hormones might be the potential signaling molecules that mediate root-to-shoot bystander signaling.

JA and its bioactive derivatives are collectively referred as to jasmonates, and play important roles in plant growth and development [36]. Synthesis of the JA begins with α-linolenic acid liberated from membrane phospholipids. The linolenic acid is first oxygenated by lipoxigenase (LOX) to form 13(*S*)-hydroxylinolenic acid (13-HPOT), which is then converted to 12-oxo-phytodeinoic acid (12-OPDA) by allene oxide synthase (AOS) and allene oxide cyclase (AOC). The 12-OPDA subsequently undergoes a reduction and three cycles of β-oxidation in the peroxisomes to produce JA [37,38], which is further modified in the cytosol to produce various JA derivatives, such as methyl jasmonate (MeJA) [38–41]. In the subsequent signaling cascades, JA is first activated by its conjugation to L-isoleucine by a JA-amino synthetase encoded by the *JASMONATE RESISTANT 1* (*JAR1*) gene. The resultant JA-Ile binds to the COI1-JAZ complex to promote degradation of the JAZ proteins, which initiates expression of down-stream JA response genes by freeing their transcription factors [42]. Mutation of the *JAR1* gene impairs JA perception and signaling cascades in plants, which exhibit decreased sensitivity to exogenous JA [43]. Although JA has been reported to act as a long-distance systemic signal transducer in response to biotic and abiotic stress [44–46], there is yet no evidence showing that JA signals can mediate RIBE in plants.

In the present study, we adopted *A. thaliana* mutants deficient in JA biosynthesis (*aos*) and signaling cascades (*jar1-1*), and investigated whether the JA signal pathway could mediate RIBE in plants. The results showed that deficiencies in both processes blocked the induction of bystander genetic and epigenetic effects, suggesting an important role of the JA signal pathway in mediating RIBE in plants. Using the root micro-grafting technique, the JA signal pathway was shown to participate in both the generation of bystander signals in irradiated root cells and radiation responses in bystander aerial tissues. The over-accumulation of endogenous JA suppressed RIBE-mediated HR repair, but up-regulated the non-homologous end joining (NHEJ) mechanism.

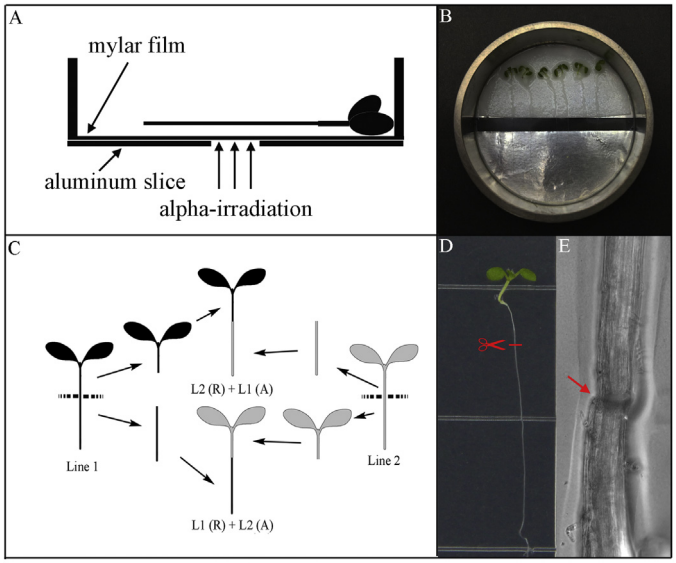

Fig 1. Root-localized irradiation and root micro-grafting of *Arabidopsis thaliana* seedlings. (A) Schematic of the root-localized irradiation of *A. thaliana* seedling with alpha particles; (B) positioning of *A. thaliana* seedlings on the radiation dish, with 100-μm-thick aluminum shielding the aerial parts of plants from alpha irradiation; (C) Schematic procedure of root micro-grafting of *A. thaliana* seedling; (D) The seedlings prepared for root micro-grafting, the red line indicates the cutting position for root grafting; (F) The graft junction at 24 h after root grafting, as indicated by red arrow.

2. Materials and methods

2.1. *Transgenic* A. thaliana *lines and plant growth*

A. thaliana line 15-6# carrying the *AtRAD54 promoter-GFP+ GUS* construct was presented by Dr. Seiichi Toki (Plant Genetic Engineering Research Unit, National Institute of Agrobiological Sciences, Japan) [47]. The line *fatty acid oxygenation up-regulated 2 (fou2)*, in which a missense mutation in the *Two Pore Channel 1* (*TPC1*) gene up-regulates expression of the *LOX* and *AOS* genes [48], was kindly donated by Prof. Edward Farmer (University of Lausanne, Switzerland). *A. thaliana* lines *aos* and *jar1-1* are mutants with loss of function of the *AOS* (CYP74A) and *JAR1* genes, respectively [49,50]. *A. thaliana* line A1 is transgenic for two copies of the *pAOS:uidA* reporter [51]. The *A. thaliana* wild-type (Col-0), A1, *aos*, and *jar1-1* lines were obtained from the NASC (Nottingham *Arabidopsis* Stock Centre, UK).

Surface-sterilized seeds of *A. thaliana* were sown on growth medium [1 × Murashige and Skoog (MS) mineral salts, agar at 0.8% (w/v), and sucrose at 1% (w/v)] in a square Petri dish. After 48 h of jarovization at 4 °C, the Petri dish was placed in a growth chamber at 22 °C with continuous illumination of approximately 100 $\mu Mm^2\ s^{-1}$ in a vertical orientation so that the roots would grow along the agar surface.