Expression Changes of Genes Related to Chromosome Remodeling Caused by Implantation with Low-energy N^+ Beam in Rice

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Abstract: In order to investigate expression changes of genes related to chromosome remodeling induced by implantation with low-energy N^+ beam in rice, the differentially expressed genes related to chromosome remodeling in treated samples (6×10^{17} N^+/cm^2), were screened using Agilent gene chip. The results showed that, in the treated samples, 1 out of 30 genes related to histone deacetylase was up-regulated. Expressions of 48 genes related to histone acetyltransferases (HATs), two SNF2-related domain containing proteins and one SWIM Zn-finger domain containing protein were up-regulated, one SWIB/MDM2 domain containing protein and one TAFII55 protein conserved region domain containing protein were down-regulated, expression of 3 GCN5-related N-acetyltransferase domain containing proteins were significantly different, in which one was up-regulated and two down-regulated. There are several conserved protein domains related to chromosome remodeling on the Microarray, Expressions of 2 of 38 bromodomain related genes were changed of which one is up-regulated and the other down-regulated. Expressions of 2 of 7 chromodomain related genes and 8 of 36 SET related genes were up-regulated. Besides, one expression of 14 LSD1 type domain containing protein was significantly up-regulated. These findings suggest that the expression changes of genes related to chromosome remodeling may regulate the expression of certain genes responding to ion implantation, and induce the biological effect of the rice implanted by ion beam.

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1. Introduction

Higher plant cell differentiation and development was controlled by the activation or silence of specific genes on the time and space, this expression pattern changes required chromatin structure reconstruction of the DNA promoters and other regulatory regions. Chromatin reconstruction led to gene activation or silence through promoting the "open" or "off" of chromatin configuration (Huang, 2008). Chromatin remodeling is achieved through ATP dependent physical modifications and covalent chemical reactions. ATP dependent chromatin remodeling factors use the energy of ATP hydrolysis to introduce superhelical torsion into DNA and to alter the rotational phasing of the DNA on the surface of the histone octamer, thus to increase the accessibility of the nucleosomal DNA to transcription factors and restriction enzymes(YA, 2011). Covalent chemical reactions dependent chromatin remodeling is the various chemical post-translational modification of histone amino-terminal, it is an important epigenetic regulation for chromatin structure and gene activity (Ng, 2000). The level of chromatin structure modification have an effect on genes expression, it is an important genetic basis for epigenetic phenomenon.

There are two gene clusters related to chromosome remodeling. Su(var) (suppresses variegation) proteins induce chromatin silence, including histone deacetylases (HDACs), protein phosphatases (PPTases), S-adenosylmethionine synthetase, heterochromatin protein HP1 and so on. On the contrary, E(var) (enhances variegation) proteins enhance the activity of nucleosome, including ATP-dependent nucleosome remodeling elements, such as SWI/SNF， brahma complex and so on. Gene products of Su (var) and E (var) also include some conserved protein domains, such as bromo-, chromo, SET, and all that (Li, 2010).

In recent years, it has become an important means to research plant genetics and breeding, growth and development, stress response and other biological effects using low energy N^+ beam to induce plant seeds (Zuo, 2003). In this study, the germination rate, dry weight, average vigor index of rice seeds have significantly improved by adopting the above method. Total mixed RNAs from the promoted-growth rice seedlings were isolated, hybridized with the Agilent GeneChip, which is genome expression chip. The data analysis showed that, in the treated samples (low

energy N^+ beam induced), the expressions of genes related to chromosome remodeling were significantly improved in comparison to untreated samples. We analyzed the different expression of genes related to chromosome remodeling between treated samples and untreated samples, to investigate the inherent connection between their expressions and phenotype changes of the treated rice.

2. Plant materials

Dry seeds of rice cultivar Xindao-18 (*Oryza sativa L. ssp. japonica*) were used in ion implantation. After being implantation of the ion beam, all seeds were planted on sterile medium with 0.8% agar (Sigma) in a climate chamber under the dark at 28℃for 96-hours. Then the seedlings were randomly divided into two groups, one group was used to RNA isolation, while the other was used to evaluate seed vigor index from the 10-days-old seedlings grown in a climate chamber under a 12-hr dark/12-hr light cycle at 28℃.

3. Methods

Implantation of low-energy N^+ ion beam and investigation of simplified seed vigor index

The low-energy ion beam implantation of seeds was performed with Ion Beam Bioengineering Facility (UIL.0.512, TNV. Russia). For implantation, germs of seeds (62 days after harvesting) were upward to the coming-energetic ion beam and endosperms of seeds were downwardly immersed into the polyfoam fixed in the sample discs and then put into target chamber of the facility. When the vacuum situation of target chamber was below 10^{-2} Pa, the seeds in sample disc were implanted with low-energy (40 KeV) N^+ with the ion fluencies: 6×10^{17} N^+/cm^2. The control seeds incubated under normal condition. After implantation, all seeds were planted on sterile medium with 0.8% agar (Sigma) in a climate chamber under the dark at 28℃. Two hundred seeds were implanted in each experiment, with three replicates. The germination rate (from one hundred seeds) was investigated from the 10-days-old seedlings, and the seedlings were oven dried at 80℃ for 12h to investigate the dry weight.

Germination rate = Number of the seedlings/total seeds×100%

Simplified seed vigor index =Drought weight of the seedlings × Germination rate

Data were pooled from three independent experiments.

RNA extraction

Total mixed RNAs from the promoted-growth rice seedlings were isolated using RNA plant reagents (Tiangen Biotech) and purified by use of the RNeasy Plant Kit (Qiagen). The yield and purity of RNA were determined spectrophotometrically (Nanodrop ND1000). Thirty uniform rice seedlings were used to extract the total RNA to construct the RNA pool of a biological replicate. So, at least 6 total RNA pools were constructed, including 3 control samples, 3 samples implanted by ion implantation.

Agilent GeneChip hybridization and data analysis

The Agilent GeneChip hybridization (Agilent Rice Oligo Microarray (4×44K) Genome Array) and raw data analysis were carried out by the *ShanghaiBio Company Ltd.*, including the procedures for cDNA and cRNA synthesis, cRNA Cy3 fluorescence labeling (GE healthcare PA13105), hybridization (Agilent G2545A), washing, scanning (Agilent G2565BA Microarray Scanner System), data collection and normalization. This experiment was performed three times, resulting in three biological replication samples for ion fluence for the significant statistics.

To screen the differentially expressed transcripts of genes related to chromosome remodeling between the implanted samples and the controls, we collect the expression signals, sorted the differentially expressed transcripts data in Excel. Based on the statistics analysis, a transcript was considered significantly up-or down regulated if it met all of the following criteria: 1) showed statistically significant differential expression at adjusted p value < 0.05; 2) had a cut-off value a 1.5-fold change; 3) had "present" calls on all of the three replicates samples for the controls and/or the implantation.

4. Results

Simplified vigor index

The results (Table1) showed that implantation of the N^+ beam (6×10^{17} N^+/cm^2) enhanced the vigor index ($P < 0.05$). In order to investigate the important molecular mechanism in encouraged rice seedlings responding to ion-beam implantation, we isolated the total mixed RNA from the samples underlying the exposure with ion-beam implantation influx: 6×10^{17} N^+/cm^2 for the Agilent GeneChip hybridization. (Simplified vigor index was investigated using the 10-day rice seedlings in each independent experiment replicates. Fluence referring to 6×10^{17} N^+/cm^2; STD, standard deviation.)

Differentially expressed transcripts of HDAC1

OsHDAC1 encodes a histone deacetylase. The higher the degree of histone acetylation for gene promoter is, the greater the activity of gene expression is. *OsHDAC1* play an important role in regulating gene expression on the epigenetic through control histone deacetylation of gene promoter. There are 30 genes related to histone deacetylase on the Microarray, one of them is differentially expressed and up-regulated in the treated samples (Table2).

Differentially expressed transcripts of HATs

Histone acetyltransferases (HATs) have been shown as positive regulators in eukaryotic transcription. The histone acetyltransferases are divided into five families.

These include the Gcn5-related acetyltranserases (GNATs), the MYST-related HATs, p300/CBP HATs, the general transcription factor HATs, which include the TFIID subunit TAF250, and the nuclear hormone-related HATs SRC1 and ACTR (SRC3) (Torchia, 1998). There are 48 genes related to histone deacetylase on the Microarray, 8 of them are differentially expressed in the treated samples (Table3).

Differentially expressed transcripts of genes about conserved protein domains related to chromosome remodeling

Bromodomains, evolutionarily conserved functional domains, can specifically interact with acetyl-lysine peptides to regulate histone acetylation through inhibiting HATs activity at specific sites involving in changes in chromatin structure and transcriptional regulation (Chen, 2001). The Chromatin Organization Modifier (Chromo) domain is defined as a 30~70 amino acid residue protein module found in many proteins involved in the assembly of protein complexes on chromatin. Chromo domains promote protein binding to methylated lysines in the tail region of histone H3. Chromo domains can function individually or in tandem, as with CHD1, to recognize specific methylated Histone tails (Jacobs, 2002). SET (Su (var), Enhancer of zeste, and Trithorax) domain protein family members share the conserved SET domain. They participate in protein methylation, chromosome structure adjustment, and gene expression regulation, and play important roles in plant development (Zhang, 2009). There are several conserved protein domains related to chromosome remodeling on the Microarray, 2 of 38 bromodomain related genes have significant differences expression, one is up-regulated and the other down-regulated. Expressions of 2 of 7 chromodomain related genes and 8 of 36 SET related genes are significantly up-regulated (Table4).

Differentially expressed transcripts of LSD1

LSD1-like proteins are a family of plant-specific transcription factors that contain a specific class of C2C2 type zinc finger domain. Two members of this family have been identified and proved to control plant programmed cell death (PCD) in *Arabidopsis*. Southern blot indicated that *OsLSD1* is a single-copy gene in rice. Furthermore, the *OsLSD1* gene was expressed constitutively in rice root, stem and leaf (Wang, 2005). There are 14 genes related to LSD1 on the Microarray, of which one is differentially expressed and up-regulated in the treated samples (Table5).

Table1. Simplified vigor index of implanted seeds

Samples	Germination rate (Mean%±STD)	Vigor index (Mean±STD)	P-value to T-test
Controls	81.85±2.31	9.55±1.69	
Fluence	88.89±0.00	14.52±1.36	0.017

Table2. Differentially expressed transcripts of *HDAC1* in rice seedlings germinating from the implanted seeds

ProbeName	Description	P-value	FC	Regulation
Os06g0583400	Histone deacetylase HDAC1	0.004	2.02	up

Table3. Differentially expressed transcripts of *HATs* in rice seedlings germinating from the implanted seeds

ProbeName	Description	p-value	FC	Regulation
Os03g0747600	GCN5-related N-acetyltransferase domain containing protein	0.042	1.60	up
Os09g0488000	GCN5-related N-acetyltransferase domain containing protein	0.030	1.78	down
Os11g0525800	GCN5-related N-acetyltransferase domain containing protein	0.023	1.60	down
Os09g0133000	SWIB/MDM2 domain containing protein	0.042	1.52	down
Os09g0480300	SWIM Zn-finger domain containing protein	0.031	1.84	up
Os02g0114000	SNF2-related domain containing protein	0.022	1.80	up
Os04g0629300	SNF2-related domain containing protein	0.037	1.84	up
Os01g0648500	TAFII55 protein conserved region domain containing protein	0.014	1.83	down

Table4. Differentially expressed transcripts of genes about conserved protein domains related to chromosome remodeling

ProbeName	Description	P-value	FC	Regulation
Os02g0699900	Bromodomain transcription factor containing protein	0.002	1.77	down
Os02g0742000	Bromo adjacent region domain containing protein	0.004	1.72	up

Os07g0497000	Chromodomain helicase-DNA-binding protein Mi-2 homolog (dMi-2).	0.012	1.86	up
Os07g0660200	Chromodomain-helicase-DNA-binding protein, CHD-1-related.	0.001	1.47	up
Os08g0180100	Nuclear protein SET domain containing protein.	0.012	2.02	up
Os05g0490700	SET domain protein SDG111	0.017	1.86	up
Os09g0556700	Nuclear protein SET domain containing protein	0.031	1.56	up
Os02g0708600	Nuclear protein SET domain containing protein	0.008	1.53	up
Os02g0725200	Nuclear protein SET domain containing protein	0.008	1.51	up
Os04g0423600	Nuclear protein SET domain containing protein	0.001	1.48	up
Os09g0134500	Trithorax-like protein 1	0.013	1.52	up
Os12g0613200	Trithorax protein.	0.017	1.45	up

Table5. Differentially expressed transcripts of *LSD1* in rice seedlings germinating from the implanted seeds

ProbeName	Description	P-value	FC	Regulation
Os08g0223700	Zn-finger, LSD1 type domain containing protein	0.034	1.55	up

5. Discussion and Conclusion

Histone deacetylases (HDACs), as negative regulators in eukaryotic transcription, modulate chromatin structure and transcription. These results demonstrate that OsHDAC1 overexpression in transgenic cells both yields enzymatically active HDAC complexes and induces changes in histone acetylation *in vivo*. Its overexpression leads to a range of novel phenotypes, involving seedling root growth (Chung, 2009) and growth rate increased and plant architecture altered, which suggests that OsHDAC1 functions in the genome-wide programming of gene expression (Jang, 2003). In this experiment, the up-regulation of *OsHDAC1* may be one of the reasons for significant increase of the seeds vigor index.

In most cases, histone acetyltransferase exist as a member of multisubunit complex. Gcn5, as part of the SAGA, ADA or HAT–A2 complexes, acetylates nucleosomes *in vitro* (Sendra, 2000). The Gcn5-related N-acetyltransferases (GNAT) as positive regulators in eukaryotic transcription, catalyze the transfer of the acetyl from the CoA donor to a primary amine of the acceptor. The SWI/SNF family of complexes utilizes the energy of ATP hydrolysis to remodel chromatin structures, thereby allowing transcription factors to gain access to DNA. SWIB domain is a conservative structure protein found in BAFb (one component of The SWI/SNF family of complexes). The SWIB and the MDM2 domains are homologous and share a common fold (Bennett-Lovsey, 2002). Zinc finger proteins are the most abundant transcription factors in eukaryotic genome, it play a key role in regulating plant defense gene expression and resistance reactions. SWIM (SWI2/SNF2 and MuDR) zinc-binding domain, which is found in a variety of prokaryotic and eukaryotic proteins (Laity, 2001). SNF2-related domain is found in proteins involved in a variety of processes including transcription regulation, DNA repair, DNA recombination, and chromatin unwinding (Eisen, 1995). SNF2 functions as the ATPase component of the SNF2/SWI multisubunit complex, which utilizes energy derived from ATP hydrolysis to disrupt histone-DNA interactions, resulting in the increased accessibility of DNA to transcription factors. The general transcription factor, TFIID, consists of the TATA-binding protein (TBP) associated with a series of TBP-associated factors (TAFs) that together participate in the assembly of the transcription preinitiation complex. TAF(II)55, as the TFIID component, binding to TAF(II)250 inhibits its AT activity. Importantly, the addition of recombinant TAF(II)55 to *in vitro* transcription assay inhibits transcription of TAF(II)250-dependent MHC class I. Thus, TAF(II)55 is capable of regulating function of TAF(II)250 by modulating its AT activity (Gegonne, 2001).

Bromodomains are evolutionarily conserved functional domains and can specifically interact with acetyl-lysine peptides to regulate histone acetylation through inhibiting HATs activity at specific sites. Bromodomain may be associated with acetylation of gene activation too, because almost all transcription-related HATs contain Bromodomain (Chen, 2001). Chromodomain helicase-DNA-binding protein (Mi-2/CHD) is subfamily of Chromatin-remodeling complexes (CRCs), which mediate ATP-dependent alterations of DNA–histone contacts and provide essential links between signaling pathways and the chromatin-based control of transcription, replication, repair, and recombination (Tomasz J. Sarnowski, 2005). EST expression data analysis showed that the expression of rice SET structural genes were the most abundant in callus, secondly in bud, which indicates that SET domain

genes may have a very close relationship with rice development (Zhang, 2009).

Zinc finger LSD1 plays a negative role in regulating plant PCD and hypersensitive response, as a positive factor in callus differentiation. Plants over-expressing LSD1 show increased content of chlorophyll b and enhanced resistance to virulent rice blast fungus. Callus over-expressing LSD1 shows accelerated differentiation and plant regeneration (Wang, 2005).

It can influence each other between the different modifications of histone, that is, one modification can often accelerate or inhibit the another modification (Wang, 2001). Different modifications can induce a special chromatin state in the manner of combine or order. Chromatin structure often changes due to the modification of various complexes, affecting DNA replication, recombination, repair, transcriptional control and so on (Huang, 2008).

Low-energy ion beam implantation is characterized as limited physiological damages, wide mutation spectrum in present generation, and the partial of phenotypic variation in the present generation can be inhered to offspring. However, many phenotypic variations did not genetically occur in the following generation, such as M4, M5 generation. As a review (Li, 2007) showed that ion beam implantation can induce DNA methylation, normal chromosome segregation, and early segregation. So we thought that low-energy ion beam implantation can induce epigenetic inherence and phenotypic variation in implanted present generation (M1). However, little information was attempted to investigate the likely molecular mechanism on epigenetic inherence caused by implantation of ion beam. This study should provide new insights into the further understanding of molecular mechanism on ion-beam implantation biological effects.

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