Exploring a Novel Genomic Safe-haven Site in the Human Pathogenic Mould Aspergillus fumigatus

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ABSTRACT

Development of novel molecular tools is necessary to fully explore the molecular landscape of the pathogenicity of A. fumigatus. In this study, we identified a new genomic safe-haven site that we term SH-aft4 at the site of an inactive Tc1/mariner type transposable element (Panel A). Our analyses demonstrate that the deletion of the aft4 element as well as the expression of a transgene construct from the aft4 locus do not have any significant impact on the growth characteristics (Panel B) and the pathogenic properties (Panel C) of A. fumigatus. We also demonstrate that the aft4 locus has a great potential to provide a robust integration site for expression of a transgenic construct in combination with the CRISPR-Cas9 mediated genome-editing system (Panel D). Furthermore, we show that SH-aft4 is highly conserved in the genomes of a large number of clinical and environmental isolates of A. fumigatus (Panel E). Our results strongly suggest that SH-aft4 locus can serve as a novel molecular tool for genetic manipulation of A. fumigatus to aid functional genomics studies of this important human fungal pathogen.

Identification of the aft4 locus, encoding an inactive transposable element, as a potential genomic safe-haven site

The aft4 locus was identified as a unique 1.3 kb nucleotide region with a moderate homology to the Fusarium oxysporum impala transposon. The aft4 element is present in the genome of the A. fumigatus isolates in common laboratory use. The full-length ORF of the aft4 transposase appear to have been genetically inactivated by several mutations but it lies in a transcriptionally active genomic region.

Deletion of the inactivated aft4 element does not have significant impact on growth characteristics

(a) Schematic representation of the aft4 locus encoding a putative inactivated Tc1/mariner type transposable element. The full-length ORF can be generated from the inactivated aft4 by introducing three nucleotide changes (T(43)A, A(44)G, G(503)C. (b) Dot plot analysis of the 20-kbp region surrounding the aft4 element between A. fumigatus AFUBT00009191 and AFUBT00014163. (c) RNA-expression profiles of the aft4 locus in A. fumigatus AFUBT00014163 indifferent culture conditions.

The potential of the aft4 locus as a transgene expression site for functional genomics applications

We examined the potential of SH-aft4 as a safe-haven site for functional genomics studies in A. fumigatus by expressing a nuclear targeted yellow fluorescent protein (NLS-Venus) as an example. Our results demonstrate that the capability of SH-aft4 for an efficient transgene integration, especially in combination with the CRISPR-Cas9 transformation system. Moreover, we are able to effectively express a transgene construct from SH-aft4 under the control of a promoter which regulates the expression of the transcription factor hpx in an iron independent manner.

The SH-aft4 locus is conserved as a single-copy element in a large subset of clinical and environmental isolates

Our bioinformatic analysis of the genome of 234 different A. fumigatus isolates that include 159 clinical and 75 environmental isolates revealed that the majority of the isolates possess SH-aft4 as a single copy element. This indicates that SH-aft4 can be used as a universal safe-haven site for most of A. fumigatus isolates.