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The transcription factor SKN-1 and detoxification gene *ugt-22* alter albendazole efficacy in *Caenorhabditis elegans*

Pauline Fontaine, Keith Choe*

Department of Biology, University of Florida, Gainesville, FL 32611, USA

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ABSTRACT

Parasitic nematodes infect over 1/4 th of the human population and are a major burden on livestock and crop production. Benzimidazole class anthelmintics are widely used to treat infections, but resistance is a widespread problem. Mutation of genes encoding the benzimidazole target β-tubulin is a well-established mechanism of resistance, but recent evidence suggests that metabolism of the drugs may also occur. Our objective was to investigate contributions of the detoxification-response transcription factor SKN-1 to anthelmintic drug resistance using *C. elegans*. We find that *skn-1* mutations alter EC_{50} of the common benzimidazole albendazole in motility assays by 1.5–1.7 fold. We also identify *ugt-22* as a detoxification gene associated with SKN-1 that influences albendazole efficacy. Mutation and overexpression of *ugt-22* alter albendazole EC_{50} by 2.3–2.5-fold. The influence of a nematode UGT on albendazole efficacy is consistent with recent studies demonstrating glucose conjugation of benzimidazoles.

1. Introduction

Parasitic nematodes infect a fourth of the world's human population (Caffrey, 2012) causing high global morbidity and mortality (Pullan et al., 2014; Torgerson et al., 2015). They also threaten agricultural and companion animals, as well as crop production causing over $100 billion losses in crop yield per year (Jasmer et al., 2003). Control of parasitic worms relies mainly on the use of a few major classes of anthelmintics, including macrocyclic lactones, imidazothiazoles, tetrahydropyrimidimes, and benzimidazoles. Benzimidazoles are the most widely used anthelmintics, with albendazole being recommended by the World Health Organization for community-wide treatment for soil-transmitted helminthiases (Anderson et al., 2014). By binding to β-tubulin BEN-1, and inhibiting microtubule polymerization (Lacey, 1990), benzimidazole drugs impair many processes in the model nematode *Caenorhabditis elegans* including body morphology and motility (Driscoll et al., 1989; Holden-Dye and Walker, 2014; Spence et al., 1982).

Nematodes have now evolved resistance to most anthelmintics, threatening sustainable control in agriculture and humans. Resistance to all three major classes of anthelmintics has been documented in parasitic nematodes and multidrug resistance can evolve in *C. elegans* under anthelmintic selection (Garcia et al., 2016; James and Davey, 2009; Ramos et al., 2016). Benzimidazole resistance is the most widespread, has been the most studied at the molecular level (Furtado et al., 2016), and resistance is emerging in human parasites (Krucken et al., 2017; Soukhathammavong et al., 2012; Vercruysse et al., 2011). Two general mechanisms have been shown to be associated with anthelmintic resistance. Mutations in genes encoding drug targets, including the benzimidazole β-tubulin target (Lacey, 1990), confer strong resistance in *C. elegans* (Driscoll et al., 1989; Lewis et al., 1980) and parasitic nematodes (Furtado et al., 2016). Evidence for anthelmintic drug biotransformation has also been accumulating recently (James and Davey, 2009; Vokral et al., 2012, 2013).

Detoxification of exogenous small molecules is a conserved metabolic process that occurs in three inter-dependent phases. In phase I, the drug is modified to introduce or reveal hydrophilic groups, which serve as anchors for phase II conjugation reactions to water-soluble moieties such as glucose and glutathione. The resulting conjugated metabolite is then pumped out of cells by phase III transporter proteins. Phase I enzymes include cytochrome P450s (CYPs) and short-chain dehydrogenases/reductases, and phase II reactions involve glutathione-S-transferases (GSTs) and UDP-glycosyltransferases (UGTs). Phase III ATP-binding cassette (ABC) transporters are efflux pumps. Benzimidazole resistance has been shown to be associated with increased expression or activity of detoxification genes and enzymes in free-living and parasitic nematodes (Jones et al., 2015; Vokral et al., 2012, 2013). However, genetic and molecular determinants of benzimidazole anthelmintic biotransformation remain largely unknown in nematodes.

* Corresponding author. Department of Biology and Genetics Institute, University of Florida, Newell Drive, Gainesville, FL 32611, USA.
E-mail address: kchoe@ufl.edu (K. Choe).

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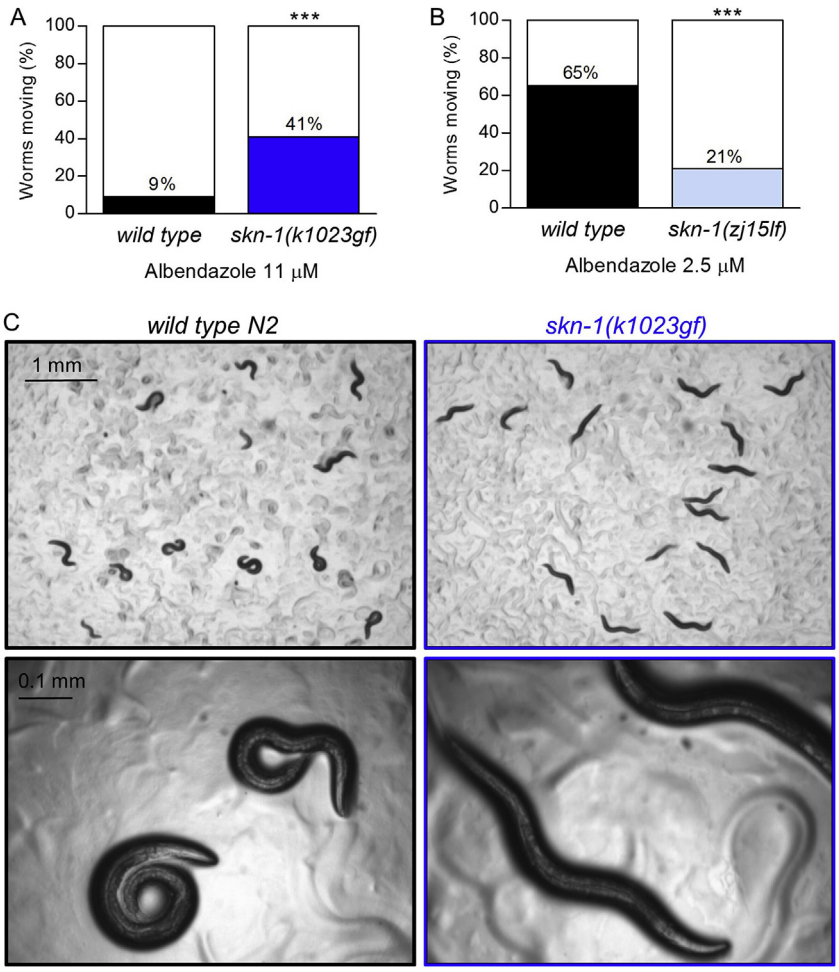

Fig. 1. *skn-1* mutations alter albendazole efficacy. (A) Percent long-term spontaneous motility of adult *wild type* N2 and *skn-1(k1023gf) (gf,* gain-of-function) worms exposed to 11 μM albendazole for 3 days. (B) Percent long-term spontaneous motility of adult *wild type* N2 and *skn-1(zj15lf)* (*lf*, loss-of-function) worms exposed to 2.5 μM albendazole for 3 days. (A and B) $n > 150$ worms per strain. ***$P < 0.001$ by Chi-square analysis. (C) *Wild type* N2 and *skn-1(k1023gf)* worms after 3 days on albendazole 11 μM plates.

The cap-n-collar (CNC) protein SKN-1 belongs to a family of basic region leucine zipper (bZIP) transcription factors that regulate expression of xenobiotic detoxification genes in *C. elegans*, *Drosophila,* and mammals (An and Blackwell, 2003; Choe et al., 2012). In *C. elegans,* SKN-1 promotes resistance to pro-oxidants and electrophiles by regulating numerous genes predicted to promote glutathione synthesis and small molecule detoxification (Choe et al., 2009, 2012; Oliveira et al., 2009; Park et al., 2009; Peddibhotla et al., 2015; Tang and Choe, 2015). SKN-1 homologs are found throughout the nematode phylum (Choe et al., 2012), but no studies have investigated them in the context of anthelmintics.

The free-living nematode *C. elegans* has been used to identify molecular targets of anthelmintics, functionally characterize drug targets, and identify molecular mechanisms of resistance (Driscoll et al., 1989; Janssen et al., 2013; Keiser, 2015). Using genetic manipulations in *C. elegans*, we show that SKN-1 influences efficacy of the common benzimidazole albendazole. Genetic manipulation of a detoxification gene associated with SKN-1 activation, *ugt-22*, also influences efficacy of albendazole. UGT-22 belongs to a group of rapidly evolving and expanding UGT protein family members that is shared with the clade V intestinal parasite *Haemonchus contortus*.

2. Materials and methods

2.1. C. elegans strains used

The following previously prepared strains were used: *wild type* N2 Bristol, QV212 *skn-1(k1023)*, QV225 *skn-1(zj15)*, CB3474 *ben-1(e1880)*, VC30084 *ugt-22(gk411724) IV*, and DR107 *unc-26(e205);dpy-4(e1166)* IV. The following transgenic lines were generated: QV303 *qvEx132*, QV304 *qvEx133*, and QV311 *qvEx140* were injected with [*ugt-22p::ugt-22 gDNA::ugt-22 3′UTR*; *myo-2p::tdTomato*; pGC31]. QV302 *qvEX131*, QV305 *qvEx134*, and QV306 *qvEx135* were injected with [*myo-2p::tdTomato*; pGC31]. QV308 *ugt-22(gk411724); qvEx137*, QV309 *ugt-22(gk411724);qvEx138*, and QV312 *ugt-22(gk411724)*; *qvEx141* were injected with [*ugt-22p::ugt-22 gDNA*:*ugt-22* 3′UTR; *myo-2p::tdTomato*; pGC31]. Worms were cultured at 20 °C (Brenner, 1974) unless otherwise stated. Table S1 lists the names, alleles, and functions of all strains used in the present study.

2.2. Outcrossing of ugt-22(gk411724)

A million mutation project *ugt-22(gk411724)* IV allele carrying a nonsense mutation was outcrossed five times to DR107 *unc-26(e205);dpy-4(e1166)* IV resulting in strain QV300 *ugt-22(gk411724)*, sometimes referred to as *ugt-22(gk411724lf)* mutant worms for simplicity. Homozygosity was verified by restriction digestion and sequencing