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ORIGINAL RESEARCH

Parkin Somatic Mutations Link Melanoma and Parkinson's Disease

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ABSTRACT

Epidemiological studies suggest a direct link between melanoma and Parkinson's disease (PD); however, the underlying molecular basis is unknown. Since mutations in *Parkin* are the major driver of early-onset PD and Parkin was recently reported to play a role in cancer development, we hypothesized that Parkin links melanoma and PD. By analyzing whole exome/genome sequencing of *Parkin* from 246 melanoma patients, we identified five non-synonymous mutations, three synonymous mutations, and one splice region variant in *Parkin* in 3.6% of the samples. *In vitro* analysis showed that wild-type Parkin plays a tumor suppressive role in melanoma development resulting in cell-cycle arrest, reduction of metabolic activity, and apoptosis. Using a mass spectrometry-based analysis, we identified potential Parkin substrates in melanoma and generated a functional protein association network. The activity of mutated Parkin was assessed by protein structure modeling and examination of Parkin E3 ligase activity. The Parkin-E28K mutation impairs Parkin ubiquitination activity and abolishes its tumor suppressive effect. Taken together, our analysis of genomic sequence and *in vitro* data indicate that Parkin is a potential link between melanoma and Parkinson's disease. Our findings suggest new approaches for early diagnosis and treatment against both diseases.

KEYWORDS: Melanoma; Parkinson's disease; Parkin; Mutation

INTRODUCTION

Melanoma, a melanocytic neoplasm, is responsible for 80% of skin cancer mortality (Flaherty et al., 2012). Parkinson's disease (PD) is the most common neurodegenerative movement disorder; PD affects 1% of the population above 60 years of age (Veeriah et al., 2010; Pan et al., 2011). Melanoma and PD seem to have very different, even opposite, phenotypes at the cellular level, as melanoma results from an uncontrolled, extensive proliferation of cells, whereas PD is characterized by degeneration and death of dopaminergic neurons. Intriguingly, however, epidemiological studies indicate a potential relationship between melanoma and PD (Herrero Hernandez, 2009; Bertoni et al., 2010; Inzelberg et al., 2011; Liu et al., 2011; Kareus et al., 2012), demonstrating that PD patients and their relatives have a higher frequency of melanoma than

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the general population, and *vice versa* (Herrero Hernandez, 2009; Gao et al., 2011; Liu et al., 2011). Recently, Parkin was also suggested as a genetic link between melanoma and PD (Hu et al., 2016). However, the molecular mechanism behind this link is still unknown.

Loss-of-function mutations in the *Parkin* gene (also named *PARK2*, *PRKN*, MIM#602544) are responsible for nearly 50% of early onset PD cases (Kahle and Haass, 2004; Guo et al., 2008, 2010). Somatic mutations in the *Parkin* gene are a known cause of autosomal recessive juvenile PD (MIM#600116), in which a progressive death of neurons in the substantia nigra takes place (Romani-Aumedes et al., 2014). Alterations in the *Parkin* gene are also associated with other diseases as demonstrated by MalaCards (Table S1), such as lung cancer (MIM#211980), ovarian cancer (MIM#167000) and susceptibility to leprosy (MIM#607572). Parkin is an E3 ubiquitin ligase responsible for substrate recognition in the ubiquitination process (Hershko and Ciechanover, 1998) that leads to substrate degradation in the proteasome (Hampe et al., 2006). The known substrates of Parkin include cell cycle regulators, e.g., Cyclin D1 (Yeo et al., 2012) and Cyclin E (Veeriah et al., 2010); apoptosis mediators, e.g., Bax (Johnson et al., 2012) and Bcl2 (Chen et al., 2010); proteins involved in synaptic transmission, e.g., Synaptotagmin 11 (Kahle and Haass, 2004).

Abnormal Parkin function may underlie certain cancers (Veeriah et al., 2010). The *Parkin* gene is located on chromosome 6q in the fragile site FRA6E (Wang et al., 2009), which is altered in a variety of human cancers (Letessier et al., 2007). In the absence of Parkin expression, cancer cell lines exhibit extensive proliferation and cell cycle abnormalities (Veeriah et al., 2010; Yeo et al., 2012), while re-expression of wild-type Parkin slows the growth of colorectal carcinoma (Poulogiannis et al., 2010), breast cancer (Letessier et al., 2007; Wang et al., 2009), and lung cancer (Poulogiannis et al., 2010; Veeriah et al., 2010; Yeo et al., 2012) through an unknown mechanism (Liu et al., 2011).

Since mutations in *Parkin* are the major driver of early-onset PD (Lücking et al., 2000; Kahle and Haass, 2004) and there is evidence that Parkin is a key player in cancer progression (Veeriah et al., 2010), we compiled and analyzed sequences of *Parkin* from 246 melanoma patients. We identified five melanoma-specific non-synonymous mutations in *Parkin* in exons known to carry mutations in PD patients. We showed that one of these mutations, E28K, abolished the ubiquitination activity of Parkin. We further demonstrated that Parkin is an inhibitor of cell cycle and an apoptotic cell death driver in melanoma cells, whereas Parkin-E28K abolishes Parkin tumor suppressive effects in melanoma. Finally, in our attempt to uncover the role of Parkin in melanoma, we identified potential Parkin substrates in melanoma using immunoprecipitation followed by mass spectrometry, and clustered the identified substrates by functionality analysis. Our data suggest that Parkin links between PD and melanoma and that Parkin may serve as a diagnostic marker.

RESULTS

Somatic mutations of the *Parkin* gene in human melanoma patients

To determine whether mutations in *Parkin* gene are present in melanoma, we compiled somatic mutation data from whole exome/genome sequencing sources (Berger et al., 2012; Hodis et al., 2012; Krauthammer et al., 2012; Nikolaev et al., 2012). These data and the analysis methods were previously described (Dutton-Regester et al., 2014). Our analysis revealed mutations in 3.6% of the 246 samples analyzed. There were six non-synonymous mutations, three synonymous mutations, and one splice region variant (Table 1). The Parkin protein has an N-terminal ubiquitin-like (UBL) domain, which is responsible for the recognition of proteins destined for ubiquitination. The C-terminal RING box includes two zinc fingers separated by an in-between RING (IBR) domain that is responsible for specific interaction with E2 enzymes (Imai et al., 2000; Shimura et al., 2000). A linker region connects the UBL and RING domains; this linker contains cleavage sites for caspases 1 and 8 (Shimura et al., 2000; Kahle and Haass, 2004) (Fig. 1A). The melanoma-specific mutations are shown in Fig. 1A in relation to the protein domains.

Importantly, our analysis was only performed on point mutations and small indels, and did not include large indels or mutations of exon rearrangements. Therefore, it is possible that *Parkin* mutational frequency in melanoma may be higher than 3.6%.

The *Parkin* mutations commonly carried by PD patients (Hedrich et al., 2004; Kay et al., 2010) were indexed by exon and by type of mutation (i.e., point mutations, exon rearrangements, small deletions, and amplifications) and are represented in proportions to their amount in each exon (Fig. 1A, upper panel). Known *Parkin* mutations span the entire gene. Although mutations in different regions alter different biochemical properties of the Parkin protein, apparently all mutations disrupt the ability of Parkin to degrade substrates, and thus manifest as loss-of-function mutations (Sriram et al., 2005). Interestingly, the most unstable region of the fragile site on chromosome 6q, FRA6E, is found in the region that contains exons 2 through 8 of the *Parkin* gene (Letessier et al., 2007), and this is the region where most melanoma and PD mutations are observed (Fig. 1A). Exons 2 and 7 of the gene were previously identified as mutational hot spots (Hedrich et al., 2004). Remarkably, melanoma-specific somatic mutations in Parkin appear in the same domains as the most common germline PD mutations (Fig. 1A), supporting the hypothesis of a genetic link between the two diseases.

The structure of Parkin has been determined by crystallography (Protein Data Bank ID 4k95) (Trempe et al., 2013). We used this structure to assess possible effects of the mutations observed in melanoma patients on Parkin activity (Fig. 1B). The E28K mutation is located in the helix of the UBL domain. Based on a corresponding residue in NEDD8 protein, which is conserved and crucial for interaction with its