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Recruitment of the oncoprotein v-ErbA to aggresomes

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ABSTRACT

Aggresome formation, a cellular response to misfolded protein aggregates, is linked to cancer and neurodegenerative disorders. Previously we showed that Gag-v-ErbA (v-ErbA), a retroviral variant of the thyroid hormone receptor (TRα1), accumulates in and sequesters TRα1 into cytoplasmic foci. Here, we show that foci represent v-ErbA targeting to aggresomes. v-ErbA colocalizes with aggresomal markers, proteasomes, hsp70, HDAC6, and mitochondria. Foci have hallmark characteristics of aggresomes: formation is microtubule-dependent, accelerated by proteasome inhibitors, and they disrupt intermediate filaments. Proteasome-mediated degradation is critical for clearance of v-ErbA and T_3-dependent TRα1 clearance. Our studies highlight v-ErbA's complex mode of action: the oncoprotein is highly mobile and trafficks between the nucleus, cytoplasm, and aggresome, carrying out distinct activities within each compartment. Dynamic trafficking to aggresomes contributes to the dominant negative activity of v-ErbA and may be enhanced by the viral Gag sequence. These studies provide insight into novel modes of oncogenesis across multiple cellular compartments.

1. Introduction

The retroviral Gag-v-ErbA oncoprotein ($p75^{gag\text{-}v\text{-}erb\text{-}A}$) is a highly mutated variant of the thyroid hormone receptor α1 (c-ErbA or TRα1), which is unable to bind thyroid hormone (T_3) and interferes with the action of TRα1 and the retinoic acid receptor in avian and mammalian cancer cells. Since the discovery of *v-erbA* as one of the oncogenes carried by the avian erythroblastosis virus (AEV), researchers have focused on the oncoprotein's complex mode of action in cells, with an emphasis on relating changes in amino acid sequence to its nuclear function (Beug et al., 1996; Thormeyer and Baniahmad, 1999). The amino acid sequence changes which contribute to its oncogenic properties include fusion of a portion of AEV Gag to its N-terminus, N- and C-terminal deletions, and 13 amino acid substitutions. The avian *c-erbA* gene was likely fused to *gag* either by homologous recombination within the host cell genome or during retrotranscription of *c-erbA* mRNA packaged into retrovirus particles (Sap et al., 1986). For simplicity, we refer to the Gag-v-ErbA oncoprotein as v-ErbA hereinafter.

Early on, v-ErbA dominant-negative activity was attributed to competition with TRα1 for T_3-responsive DNA elements and/or auxiliary factors involved in the transcriptional regulation of T_3-responsive genes. It is now known that oncogenic conversion of v-ErbA from its cellular homolog involves not only changes in DNA binding specificity and ligand binding properties, but also the acquisition of altered nuclear export capabilities and changes in subcellular localization (Bonamy and Allison, 2006). As part of our studies, we noted that v-ErbA and other dominant negative variants of TR have a greater cytoplasmic localization compared with the wild-type receptor and often show a punctate distribution in cytoplasmic or nuclear foci (Bonamy et al., 2005; Bunn et al., 2001; DeLong et al., 2004). Even single amino acid substitutions in TR are sufficient to shift its balance to a more cytoplasmic distribution. Previously, we showed that dominant negative variants of another TR isoform, TRβ, which carry single amino acid substitutions in the DNA-binding domain (Gly121Ala and Cys122Ala), form perinuclear cytoplasmic foci (Bunn et al., 2001). Interestingly, this distribution pattern is very similar to the pattern described for a TRα dominant negative mutant in which the entire hinge, or D, domain was deleted (Lee and Mahdavi, 1993).

Upon further analysis of v-ErbA trafficking, we made a surprising discovery. Wild-type TRα1 is primarily nuclear at steady-state (Bunn et al., 2001); however, when co-expressed with v-ErbA

Abbreviations: TRα1, thyroid hormone receptor α1; T_3, thyroid hormone; AEV, avian erythroblastosis virus; TGF-β, transforming growth factor-β; LMB, leptomycin B; MTOC, microtubule organizing center; HDAC6, histone deacetylase 6; CFTR, cystic fibrosis transmembrane conductance regulator.

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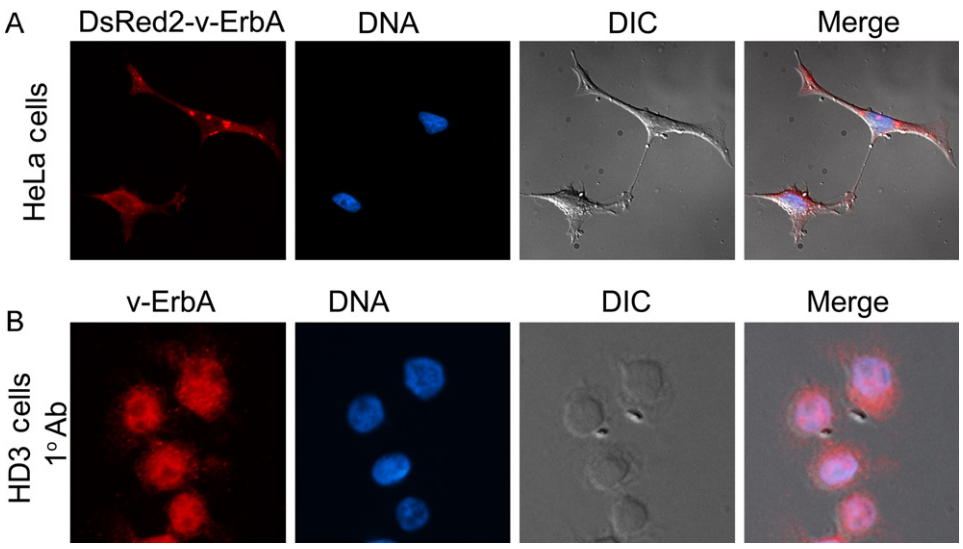

Fig. 1. A subpopulation of the oncoprotein v-ErbA localizes to punctate foci in erythroblasts transformed with AEV. (A) The distribution of v-ErbA in transfected HeLa cells ranges from whole cell (with a nuclear subpopulation) to a mainly cytoplasmic distribution, localized in punctate, perinuclear foci. HeLa cells were transfected with DsRed2-v-ErbA expression vectors. Twenty-four hours post-transfection, cells were fixed and analyzed by fluorescence and differential interference contrast (DIC) microscopy. Nuclei were stained for DNA with DAPI (blue). (B) Native, virally expressed v-ErbA forms cytoplasmic foci in chicken erythroblasts transformed with AEV (HD3 cells). Native v-ErbA was visualized in HD3 cells by immunostaining (red).

there is a striking and dramatic shift in the distribution pattern of TRα1 (Bonamy et al., 2005). v-ErbA dimerizes with TRα1 and the retinoid X receptor, and sequesters a significant fraction of the two nuclear receptors in the cytoplasm (Bonamy et al., 2005). These results defined a new mode of action of v-ErbA, and illustrated the importance of cellular compartmentalization in transcriptional regulation (Bonamy and Allison, 2006). Our findings were closely followed by a report defining a cytoplasmic function for v-ErbA, whereby the oncoprotein sequesters Smad4 into the cytoplasm and disrupts the transforming growth factor-β (TGF-β) pathway (Erickson and Liu, 2009). To further explore the cytoplasmic activities of v-ErbA, we sought to ascertain the nature of the cytoplasmic foci formed by a subpopulation of v-ErbA.

Newly synthesized proteins must fold correctly to become functional. When the protein is misfolded, hydrophobic residues that are normally buried in the protein's interior are exposed leading to protein aggregation. Cells have evolved quality control systems that are conserved from yeast to mammalian cells to minimize protein misfolding and prevent protein aggregation (Bagola and Sommer, 2008). Molecular chaperones such as the heat shock proteins assist in refolding misfolded proteins, and bind to and stabilize exposed hydrophobic residues thereby reducing the likelihood of protein aggregation (Bercovich et al., 1997; Dul et al., 2001; Morimoto, 2008; Schroder and Kaufman, 2005). Alternatively, misfolded and aggregated proteins are destroyed by the ubiquitin-mediated proteasome degradation pathway (Pankiv et al., 2007; Ross and Poirier, 2004; Rubinsztein, 2006) or through the autophagy-lysosome system (Iwata et al., 2005; Levine and Kroemer, 2008; Mizushima et al., 2008; Mortimore et al., 1996). Recent evidence suggests that cells have another important quality control pathway in which aggregated proteins are specifically delivered to inclusion bodies by dynein-dependent retrograde transport on microtubules. This microtubule-dependent inclusion body is called an aggresome (Garcia-Mata et al., 2002; Johnston et al., 1998; Kawaguchi et al., 2003; Kopito, 2000; Zhou et al., 2009). To promote disposal of the aggregated material, cytoplasmic aggresomes recruit chaperones and proteasomes, or trigger autophagy. In addition to waste disposal systems for the accumulation of aggregated proteins, aggresomes can also function as sites for viral replication and assembly (Wileman, 2006, 2007). Dysregulation of these mechanisms for degradation of misfolded or mutant proteins has been implicated in numerous clinical diseases, including cystic fibrosis, neurodegenerative disorders, and cancer (Heir et al., 2006; Iwata et al., 2005; Kitami et al., 2006; Ma and Lindquist, 2001; Olzmann and Chin, 2008; Riley et al., 2002; Ross and Poirier, 2004; Sha et al., 2009; Shimohata et al., 2002; Simms-Waldrip et al., 2008).

Here, we provide evidence for targeting of the oncoprotein v-ErbA to aggresomes. We have shown that v-ErbA foci display aggresomal characteristics by documenting the colocalization of v-ErbA inclusions with aggresome, proteasome, and mitochondrial markers as well as the dependence of the trafficking of these inclusions upon the dynein/dynactin motor and microtubules. Our studies offer insight into how cytoplasmic mislocalization of TRα1 by v-ErbA into aggresomes may contribute to oncogenesis. Association of a viral oncoprotein with these specialized quality control compartments provides further insight into the generality of this mechanism of aggresome trafficking for dealing with mutant proteins.

2. Results

2.1. A subpopulation of the oncoprotein v-ErbA localizes to punctate foci in erythroblasts transformed with AEV

Prior studies had shown that v-ErbA expressed in transfected mammalian cell lines has a range of distributions from whole cell (with a nuclear subpopulation) to a mainly cytoplasmic distribution, localized in punctate, perinuclear foci suggestive of aggresome-like inclusions (Bonamy et al., 2005; Bunn et al., 2001). These observations raised the question of whether this localization in transfected cells adequately recapitulates the distribution of the native, virally expressed v-ErbA. Since v-ErbA was originally isolated from chicken erythroblast cells, we used HD3 cells to investigate the distribution of native v-ErbA. HD3 cells are chicken erythroblasts transformed by AEV that express both native v-ErbA and v-ErbB (Erickson and Liu, 2009). After immunostaining with anti-v-ErbA/c-ErbA antibodies, HD3 cells were visualized by fluorescence microscopy. HD3 cells are semi-adherent cells with a rounded-up morphology and less visible cytoplasm compared with fully adherent HeLa cells (Fig. 1). Despite these differences in mor-