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Neuroanatomical Distribution of DEK Protein in Corticolimbic Circuits Associated with Learning and Memory in Adult Male and Female Mice

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Abstract—DEK, a chromatin-remodeling gene expressed in most human tissues, is known for its role in cancer biology and autoimmune diseases. DEK depletion *in vitro* reduces cellular proliferation, induces DNA damage subsequently leading to apoptosis, and down-regulates canonical Wnt/β-catenin signaling, a molecular pathway essential for learning and memory. Despite a recognized role in cancer (non-neuronal) cells, DEK expression and function is not well characterized in the central nervous system. We conducted a gene ontology analysis (ToppGene), using a cancer database to identify genes associated with DEK deficiency, which pinpointed several genes associated with cognitive-related diseases (i.e., Alzheimer's disease, presenile dementia). Based on this information, we examined DEK expression in corticolimbic structures associated with learning and memory in adult male and female mice using immunohistochemistry. DEK was expressed throughout the brain in both sexes, including the medial prefrontal cortex (prelimbic, infralimbic and dorsal peduncular). DEK was also abundant in all amygdalar subdivisions (basolateral, central and medial) and in the hippocampus including the CA1, CA2, CA3, dentate gyrus (DG), ventral subiculum and entorhinal cortex. Of note, compared to males, females had significantly higher DEK immunoreactivity in the CA1, indicating a sex difference in this region. DEK was co-expressed with neuronal and microglial markers in the CA1 and DG, whereas only a small percentage of DEK cells were in apposition to astrocytes in these areas. Given the reported inverse cellular and molecular profiles (e.g., cell survival, Wnt pathway) between cancer and Alzheimer's disease, these findings suggest a potentially important role of DEK in cognition. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Keywords: cognition, hippocampus, oncogene, learning and memory, Wnt pathway, sex differences, Alzheimer's disease.

INTRODUCTION

Aberrant expression or localization of the DEK DNA-binding protein has been associated with several diseases, including acute myeloid leukemia (Von Lindern et al., 1992; Logan et al., 2015), various types of solid tumors (Piao et al., 2014; Wang et al., 2014; Privette Vinnedge et al., 2015), and as an auto-antigen in numerous auto-immune diseases, most notably juvenile idiopathic arthritis (Sierakowska et al., 1993; Szer et al., 1994; Mor-Vaknin et al., 2011). DEK (human 6p22.3) is a unique protein, with no known homologs, that preferentially binds supercoiled and cruciform DNA *in vitro*. DEK is primarily expressed in proliferating cells, largely due to transcriptional regulation of *DEK* by the E2F family of transcription factors and steroid hormone receptors (Carro et al., 2006; Privette Vinnedge et al., 2012). Therefore, it is frequently over-expressed in solid tumors, especially melanoma (Khodadoust et al., 2009; Riveiro-Falkenbach et al., 2017), breast cancer (Privette Vinnedge et al., 2011, 2012), and human papilloma virus (HPV)-induced cancers including cervical cancer (Wise-Draper et al., 2005; Wu et al., 2008; Liu et al., 2012) and head and neck squamous cell carcinomas (Adams et al., 2015a, 2015b). DEK can be localized intracellularly

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Abbreviations: BLA, basolateral amygdaloid complex; CA, cornu ammonis; CeA, central amygdaloid complex; CNS, central nervous system; DG, dentate gyrus; DP, dorsal peduncular cortex; ENT, entorhinal cortex; GCL, granule cell layer; HNSCC, human head and neck squamous cell carcinoma; IL, infralimbic cortex; MeA, medial amygdaloid complex; ML, molecular layer; mPFC, medial prefrontal cortex; PL, polymorphic layer; PrL, prelimbic cortex; SGZ, subgranular zone; shRNA, short-hairpin RNA; SL, stratum lacunosum moleculare; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; VS, ventral subiculum.

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and extracellularly. However, its tissue-specific expression and intracellular function(s) in non-diseased tissue remain poorly defined.

Due to its unique nucleic acid-binding properties, DEK has been implicated in several cellular processes, including DNA replication, DNA repair, chromatin remodeling, transcription activation and repression, and mRNA splicing (Wise-Draper et al., 2009a; Riveiro-Falkenbach and Soengas, 2010). The characterization of DEK as an oncogene is attributed to its intracellular properties. For example, DEK localized within the nucleus is associated with DNA repair, and its overexpression is anti-apoptotic, promotes cellular proliferation, and prevents differentiation (Wise-Draper et al., 2006, 2009b; Kappes et al., 2008; Privette Vinnedge et al., 2015). In contrast, DEK deficiency induces DNA damage, likely due to insufficient DNA repair, as well as cellular senescence, and apoptosis (Kim et al., 2009; Kavanaugh et al., 2011). DEK is not only expressed intracellularly but also can be found extracellularly in biofluids, such as synovial fluid. DEK is secreted by macrophages under proinflammatory conditions where it can serve as a chemotactic molecule for neutrophils, CD8+ T lymphocytes, and natural killer (NK) cells. Furthermore, *in vitro* models using macrophages and HeLa cells demonstrated that extracellular DEK can be taken in by neighboring epithelial cells through a heparan sulfate-dependent process, and then translocate back into the nucleus to perform its chromatin modifying functions (Saha et al., 2013). High DEK levels in the extracellular space are associated with autoimmune disorders including juvenile rheumatoid arthritis, due to the synthesis of autoantibodies against DEK (Sierakowska et al., 1993; Mor-Vaknin et al., 2011).

The cellular proliferative effects of DEK in the periphery are mediated in part via the transcription of Wnt ligands and subsequent activation of the canonical Wnt/β-catenin signaling pathway (Privette Vinnedge et al., 2012, 2015). Accordingly, DEK deficiency in cancer cells and mouse embryonic fibroblasts down-regulates the canonical Wnt pathway, a critical molecular pathway for learning and memory. In the brain, Wnt proteins are essential for proper maintenance and function of the hippocampal formation (Fortress et al., 2013). Specifically, the canonical Wnt/β-catenin pathway plays a key role in synaptic plasticity and the formation of memories within the hippocampus and the amygdala (Fortress et al., 2013; Riise et al., 2015; Fortress and Frick, 2016). Although *DEK* mRNA expression has been reported in the adult human brain, with greater expression in malignant versus healthy brain tissue (Kroes et al., 2000), the neuroanatomical distribution of DEK protein in the murine adult brain has not been characterized. Given the association between DEK in the periphery with the Wnt signaling pathway, and because DEK deficiency gives rise to many of the cellular and molecular anomalies associated with cognitive dysfunction (DNA damage, apoptosis, cellular senescence), the goal of this study was to characterize DEK protein expression in brain regions associated with various forms of learning and memory (medial prefrontal cortex, hippocampus, and amygdala).

DEK is an estrogen receptor responsive target gene (Privette Vinnedge et al., 2012). As such, we examined DEK expression in both adult male and female brains. Based on the aforementioned findings, we hypothesized that DEK protein expression would be abundant in corticolimbic structures regulating learning and memory and that DEK expression would be higher in females relative to males. We report that DEK is ubiquitously expressed throughout adult male and female murine brain and that DEK is co-expressed with neurons, astrocytes, and microglia in the dentate gyrus. Indeed, we also note a sex difference in the brain, with a higher number of DEK-positive cells in the CA1 region of the hippocampus of adult female mice.

EXPERIMENTAL PROCEDURES

RNA sequencing

Previously reported RNA-Sequencing data were acquired from the NCBI Gene Expression Omnibus Series accession number GSE70462. Briefly, the human head and neck squamous cell carcinoma (HNSCC) cell line UM-SCC-1 was transduced with lentiviral pLKO.1 vector with either nontargeting control shRNA (NTsh) or DEK shRNA (DEK832; Sigma–Aldrich Mission shRNA library). Following selection in puromycin, RNA was purified and analyzed on an Illumina HiSeq2500 for single-end sequencing with 50 base pair reads (Adams et al., 2015a).

Animals

Dek knockout mice. In order to determine antibody specificity, adult female $Dek^{-/-}$ knockout (KO) mice ($n = 6$) and wild-type (WT) littermate controls ($n = 5$) obtained from Cincinnati Children's Medical Hospital were used. Dek KO mice were generated by using a targeting-construct containing 8.6 kb of genomic DNA with the *IRES-LacZ-Neo* selectable marker inserted into NsiI site in exon 6 (Wise-Draper et al., 2009a; Broxmeyer et al., 2012) and were backcrossed into a C57BL/6 background.

DEK expression in corticolimbic circuits. Male ($n = 8$) and female ($n = 8$) 12-week-old C57BL/6 mice from Jackson Laboratories (Bar Harbor, ME) were used to determine DEK expression in corticolimbic circuits associated with learning and memory.

DEK co-expression with neurons, astrocytes and microglia. A separate cohort of animals male ($n = 8$) and female ($n = 8$) 12-week-old C57BL/6 mice from Jackson Laboratories (Bar Harbor, ME) were used to determine if DEK was expressed in neurons, astrocytes, and microglia in the hippocampus. Because DEK is an estrogen receptor (ER) α target gene (Privette Vinnedge et al., 2012), the estrous phase of cycling mice was determined at the end of the study using previously published methods (Becker et al., 2005).

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