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Sven Bulterijs

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Generation and characterization of antibodies against arginine-derived advanced glycation endproducts

Tina Wang, Matthew D. Streeter, David A. Spiegel

Abstract

Although antibodies reagents have been widely employed for studying advanced glycation end-products (AGEs), these materials have been produced using complex mixtures of immunogens. Consequently, their epitope specificity remains unknown. Here we have generated the first antibodies capable of recognizing each of the three isomers of the methylglyoxal hydroimidazolones (MG-Hs) by using chemical synthesis to create homogenous immunogens. Furthermore, we have thoroughly characterized the epitope specificity of both our antibodies and that of two existing monoclonals by implementing a direct ELISA protocol employing synthetic MG-H antigens. Finally, we employed the reported anti-MG-H antibodies to the detection of MG-Hs in cellular systems using immunofluorescence microscopy. These studies have demonstrated that anti-MG-H1 and anti-MG-H3 staining is concentrated within the nucleus, while anti-MG-H2 affords only minimal signal. These observations are consistent with reported formation preferences for MG-Hs, and may suggest novel nuclear targets for non-enzymatic posttranslational modification. The antibody reagents reported herein, as well as the strategy employed for their creation, are likely to prove useful for the immunochemical study of AGEs in biological systems.

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KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis

Previous studies investigating the role of smooth muscle cells (SMCs) and macrophages in the pathogenesis of atherosclerosis have provided controversial results owing to the use of unreliable methods for clearly identifying each of these cell types. Here, using *Myh11*-CreER^{T2} ROSA floxed STOP eYFP *Apoe*$^{-/-}$ mice to perform SMC lineage tracing, we find that traditional methods for detecting SMCs based on immunostaining for SMC markers fail to detect >80% of SMC-derived cells within advanced atherosclerotic lesions. These unidentified SMC-derived cells exhibit phenotypes of other cell lineages, including macrophages and mesenchymal stem cells (MSCs). SMC-specific conditional knockout of Krüppel-like factor 4 (*Klf4*) resulted in reduced numbers of SMC-derived MSC- and macrophage-like cells, a marked reduction in lesion size, and increases in multiple indices of plaque stability, including an increase in fibrous cap thickness as compared to wild-type controls. On the basis of *in vivo* KLF4 chromatin immunoprecipitation–sequencing (ChIP-seq) analyses and studies of cholesterol-treated cultured SMCs, we identified >800 KLF4 target genes, including many that regulate pro-inflammatory responses of SMCs. Our findings indicate that the contribution of SMCs to atherosclerotic plaques has been greatly underestimated, and that KLF4-dependent transitions in SMC phenotype are critical in lesion pathogenesis.

β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis

Aging drives cognitive and regenerative impairments in the adult brain, increasing susceptibility to neurodegenerative disorders in healthy individuals[1, 2, 3, 4]. Experiments using heterochronic parabiosis, in which the circulatory systems of young and old animals are joined, indicate that circulating pro-aging factors in old blood drive aging phenotypes in the brain[5, 6]. Here we identify β2-microglobulin (B2M), a component of major histocompatibility complex class 1 (MHC I) molecules, as a circulating factor that negatively regulates cognitive and regenerative function in the adult hippocampus in an age-dependent manner. B2M is elevated in the blood of aging humans and mice, and it is increased within the hippocampus of aged mice and young heterochronic parabionts. Exogenous B2M injected systemically, or locally in the hippocampus, impairs hippocampal-dependent cognitive function and neurogenesis in young mice. The negative effects of B2M and heterochronic parabiosis are, in part, mitigated in the hippocampus of young transporter associated with antigen processing 1 (*Tap1*)-deficient mice with reduced cell surface expression of MHC I. The absence of endogenous B2M expression abrogates age-related cognitive decline and enhances neurogenesis in aged mice. Our data indicate that systemic B2M accumulation in aging blood promotes age-related cognitive dysfunction and impairs neurogenesis, in part via MHC I, suggesting that B2M may be targeted therapeutically in old age.

Systematic Aβ Analysis in *Drosophila* Reveals High Toxicity for the 1-42, 3-42 and 11-42 Peptides, and Emphasizes N- and C-Terminal Residues

Brain amyloid plaques are a hallmark of Alzheimer's disease (AD), and primarily consist of aggregated Aβ peptides. While Aβ 1-40 and Aβ 1-42 are the most abundant, a number of other Aβ peptides have also been identified. Studies have indicated differential toxicity for these various Aβ peptides, but *in vivo* toxicity has not been systematically tested. To address this issue, we generated improved transgenic *Drosophila UAS* strains expressing 11 pertinent Aβ peptides. *UAS* transgenic flies were generated by identical chromosomal insertion, hence removing any transgenic position effects, and crossed to a novel and robust *Gal4* driver line. Using this improved *Gal4/UAS* set-up, survival and activity assays revealed that Aβ 1-42 severely shortens lifespan and reduces activity. N-terminal truncated peptides were quite toxic, with 3-42 similar to 1-42, while 11-42 showed a pronounced but less severe phenotype. N-terminal mutations in 3-42 (E3A) or 11-42 (E11A) resulted in reduced toxicity for 11-42, and reduced aggregation for both variants. Strikingly, C-terminal truncation of Aβ (1-41, -40, -39, -38, -37) were non-toxic. In contrast, C-terminal extension to 1-43 resulted in reduced lifespan and activity, but not to the same extent as 1-42. Mutating residue 42 in 1-42 (A42D, A42R and A42W) greatly reduced Aβ accumulation and toxicity. Histological and biochemical analysis revealed strong correlation between *in vivo* toxicity and brain Aβ aggregate load, as well as amount of insoluble Aβ. This systematic *Drosophila in vivo* and *in vitro* analysis reveals crucial N- and C-terminal specificity for Aβ neurotoxicity and aggregation, and underscores the importance of residues 1-10 and E11, as well as a pivotal role of A42.

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