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Research Report

Genomic analysis of response to traumatic brain injury in a mouse model of Alzheimer's disease (APPsw)

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ABSTRACT

Numerous studies have shown that the β-amyloid peptide (Aβ) or β-amyloid deposits impact many processes that can contribute to neurodegeneration, ranging from immune and inflammatory processes to cell death and apoptosis, processes characteristic of both Alzheimer's disease and head injury. Human and animal studies of traumatic brain injury (TBI) have shown that Aβ production is increased acutely following injury, and there is evidence for increased amyloid deposition and risk for Alzheimer's disease following TBI. Given the poorer outcome after injury observed both in transgenic mice overproducing Aβ, as well as in humans subjected to repetitive head injury, one may conclude that the presence of elevated brain levels of Aβ, whether endogenous or as a consequence of previous injury, exacerbates many of the deleterious processes triggered by TBI. We sought to test this hypothesis by examining the genomic response to injury in wild-type mice and in transgenic mice (APPsw) overexpressing and accumulating cerebral Aβ/β-amyloid. Gene expression was investigated by microarray 24 h after controlled cortical impact (CCI) injury or sham injury in aged APPsw transgenic mice and wild-type controls. Stringent statistical analysis revealed differential expression of a total of 129 genes in the transgenic TBI vs. sham comparison and 119 genes in the wild-type TBI vs. sham comparison. Of these, only 28 genes were common to both comparisons, suggesting considerable differences in response to injury in the Alzheimer models compared to wild-type mice. We focused our analyses by creating a "genotype-dependent" data set of response to injury which contained the genes that were uniquely altered in response to injury in either wild-type or APPsw mice, as well as those which were significantly differently modulated following TBI in one genotype compared to the other. The cellular functions predicted to be influenced by these changes in gene expression thus indicate the adverse pathways triggered by increased levels of Aβ,

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Abbreviations: Aβ, beta-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; APPsw, Alzheimer's mouse model with the "Swedish" mutation; CCI, controlled cortical impact; IPA, Ingenuity Pathway Analysis; TBI, traumatic brain injury

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and the potentially favorable (recovery) pathways which are activated in wild-type mice but suppressed when Aβ levels are high. The results show that the cellular functions most influenced by the cerebral Aβ levels following TBI include inflammation, immune response, and cell death, which suggest a particular vulnerability to head injury in the Alzheimer brain.

1. Introduction

Around 5.3 million people in the United States suffer from some degree of traumatic brain injury (TBI)-related disability, and approximately 1.5 million more cases occur every year (Brain Injury Association of America http://www.biausa.org/). Disabilities resulting from a TBI depend upon the severity of the injury, the location of the injury, and the age and general health of the individual. Some common disabilities include cognitive problems as well as problems with sensory processing, communication and behavior or mental health. The nature and extent of recovery after TBI are heterogeneous and not fully explained by known demographic and injury prognostic features, suggesting that additional factors modulate the secondary injury/recovery pathways (Teasdale and Graham, 1998). In human studies, TBI has been demonstrated to result in amyloid deposits reminiscent of Alzheimer pathology (Roberts et al., 1994; Ikonomovic et al., 2004).

Repetitive TBI has particularly debilitating consequences and Alzheimer's-like cognitive deterioration is reported in such cases. The brains of individuals suffering chronic TBI, such as that incurred in a boxing career, show many neuropathological characteristics of Alzheimer's disease including neurofibrillary tangles, diffuse amyloid plaques, acetylcholine deficiency, and/or tau immunoreactivity (Jordan, 2000). In another study of 2552 retired professional American football players, there was a fivefold increase of mild cognitive impairment (MCI) diagnoses and a threefold increase of reported significant memory problems among retirees with three or more reported concussions compared with retirees with no history of concussion (Guskiewicz et al., 2005). This study also observed an earlier onset of Alzheimer's disease in the retirees than in the general American male population.

Consistent with the human studies, in rodent models of TBI a "burst" of amyloid production is observed in the brain within hours of injury (Uryu et al., 2002; Wang et al., 2007), and a worse outcome is observed with repetitive brain injury as compared to a single incident. The response to TBI has been studied in several different mouse models of Alzheimer's disease which overproduce Aβ, and in all cases the outcome (neurological and/or neuropathological) is worse than in their wild-type controls.

There are no published studies reporting the outcome of TBI in Alzheimer's patients, but anecdotal evidence from caregivers consistently reports augmentation of decline in AD patients who suffer a head injury. Adults over the age of 60 exhibit worse outcome after TBI than younger adults, Alzheimer's patients are at increased risk for falls (Morris et al., 1987), and at least half of the people in U.S. nursing homes have Alzheimer's disease or a related disorder. When these facts are considered together with the statistics on falls in nursing homes, many of which involve a blow to the head with a loss of consciousness, it is clear that the scenario of TBI in a condition of increased Aβ is likely present at an alarming level in our society.

Many of the processes triggered by TBI and contributing to neurodegeneration, such as immune and inflammatory mechanisms, cell death and apoptosis, are known to be influenced by Aβ and/or β-amyloid deposits (see for example Nakagawa et al., 1999, 2000). Together these data propose a role for Aβ in response to TBI and suggest that when head injury occurs in a situation of already elevated levels of Aβ, the TBI-triggered processes will be exacerbated.

To test this hypothesis, we have examined the genomic response to controlled cortical impact (CCI) injury in the APPsw mouse model of Alzheimer's disease and non-transgenic littermates. We hypothesized that the pre-existing elevated Aβ levels in the APPsw mice would exacerbate the deleterious processes triggered by TBI, as compared to the wild-type mice. In order to best distinguish the effects of elevated ambient Aβ levels on the brain's response to injury, our analyses focused on the genomic responses to TBI that were uniquely altered in either the APPsw or wild-type mice, and the cellular functions predicted to be affected by those changes in gene expression.

2. Results

In a comparison of genomic response to TBI between APPsw transgenic mice and wild-type mice, stringent statistical analysis revealed that, out of the ≈12,000 genes interrogated, 174 Affymetrix IDs were significantly regulated in the injured wild-type mice compared to sham controls. Using Ingenuity Pathways Analysis (IPA) software, these IDs inculcated 119 genes eligible to be mapped onto functions, pathways, and lists. In the APPsw mice, 185 IDs corresponding to 129 IPA-eligible genes were significantly regulated in response to injury (for a complete list see http://www.rfdn.org/publication-suppl/2007TBI/tables.html). Only 28 genes were common to both groups, several of which have previously been implicated in response to TBI (see discussion). Twenty-five of those 28 were similarly regulated (up or down) in both APPsw and wild-type data sets; genes HF1 and WNK were downregulated in APPsw and upregulated in wild-type, while gene NNAT was upregulated in APPsw and downregulated in wild-type.

Data mining was carried out with the IPA application. Across the entire data set, a total of 78 higher level functional groupings of genes, as well as 36 canonical pathways, were recognized by IPA. Only two of the 36 canonical pathways identified by Ingenuity were associated with *p*-values of less than 0.05. These were *Chemokine signaling* in the wild-type TBI vs. sham comparison data and *G1/S checkpoint regulation* in the transgenic TBI vs. sham comparison data. The top five higher level functional groups which were significantly modulated in the wild-type TBI vs. sham comparison were *cellular growth and proliferation*, *cellular movement*, *hematological system*