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The neuro-glial-vascular interrelations in genomic instability symptoms

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ABSTRACT

A hallmark of neurodegenerative diseases is impairment of certain aspects of "brain functionality", which is defined as the total input and output of the brain's neural circuits and networks. A given neurodegenerative disorder is characterized by affected network organization and topology, cell numbers, cellular functionality, and the interactions between neural circuits. Neuroscientists generally view neurodegenerative disorders as diseases of neuronal cells; however, recent advances suggest a role for glial cells and an impaired vascular system in the etiology of certain neurodegenerative diseases. It is now clear that brain pathology is, to a very great extent, pathology of neurons, glia and the vascular system as these determine the degree of neuronal death as well as the outcome and scale of the neurological deficit. This review article is focused on the intricate interrelations among neurons, glia, the vascular system, neuronal cells, and the DNA damage response. Here I describe various aspects of neural and glial cell fate and the vascular system in genomic instability disorders including ataxia telangiectasia (A-T) and Nijmegen breakage syndrome.

1. The DNA damage response

Protection of genome stability and integrity is essential for maintenance of cellular homeostasis and prevention of undue cell death or neoplasia (Kwei et al., 2010; Pino and Chung, 2010; Negrini et al., 2010). DNA damage caused by internal or external damaging agents is a major threat to the integrity of the cellular genome (Benfenati et al., 2009). The cellular defense system against this threat is the DNA damage response (DDR) – an elaborate signaling network activated by DNA damage that swiftly modulates many physiological processes (Ciccia and Elledge, 2010). One of the most powerful triggers of the DDR is the DNA double-strand break (DSB) (Bassing and Alt, 2004; Hartlerode and Scully, 2009). DSBs are induced by ionizing radiation, radiomimetic chemicals, or reactive oxygen species formed in the course of normal metabolism and can also result from replication fork stalling (Bassing and Alt, 2004). DSBs also accompany normal genomic transactions such as meiotic recombination and the rearrangement of the antigen receptor genes via V(D)J recombination (Bassing and Alt, 2004; Harrison and Haber, 2006). The major DSB repair pathways in eukaryotic cells are error-prone non-homologous end-joining (NHEJ) (Lieber et al., 2004) and a high-fidelity process based on homologous recombination (HR) between sister chromatids (Wyman and Kanaar, 2006). The overall cellular response to DSBs goes far beyond repair, however (Ciccia and Elledge, 2010). This broad, powerful signaling network works swiftly and vigorously to affect a large number of cellular systems (Ciccia and Elledge, 2010; Harrison and Haber, 2006; Bakkenist and Kastan, 2004; Shiloh, 2003; Shiloh, 2006).

The initial stage of the DSB response is carried out by a large, heterogeneous group of proteins dubbed *sensors* or *mediators* that are recruited to the damaged sites where they create expanding nuclear foci (Lisby and Rothstein, 2009). These proteins are involved in the recognition of the damage and in the subsequent damage processing, chromatin reorganization, and activation of the *transducers* of the DNA damage alarm. This initial response at the DSB sites is characterized by extensive changes in protein post-translational modifications such as phosphorylation (Matsuoka et al., 2007; Bennetzen et al., 2010), ubiquitylation (Ulrich and Walden, 2010; Nakada et al., 2010; Panier and Durocher, 2009), SUMOylation (Polo et al., 2010; Morris et al., 2010), and acetylation (Miller et al., 2010; Kaidi et al., 2010). One of the early and important phosphorylations at the vicinity of DSBs is that of the variant histone H2AX. Phosphorylated H2AX (γH2AX) plays an important role in anchoring damage response proteins to the damaged sites (Xie et al., 2010).

The primary transducer of the DSB alarm is the ATM protein, whose encoding gene, *ATM*, was identified in the Shiloh laboratory (Savitsky et al., 1995a,b). Its activity was subsequently identified as that of a serine-threonine kinase (Canman et al., 1998; Banin et al., 1998). In response to DSBs, ATM is rapidly activated (Canman et al., 1998; Banin et al., 1998; Bakkenist and Kastan, 2003), and it phosphorylates a plethora of key players in various damage response pathways (Shiloh, 2006; Lavin, 2007). Do DNA DSBs and

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chromatin alterations are the sole activator of ATM? Recent study by Guo et al. (2010) showed that oxidation of ATM directly induces ATM activation in the absence of DNA DSBs and the MRN complex suggesting that ATM is an important sensor of reactive oxygen species in human cells. These results further suggest that ATM has cellular functions, which are not directly connected to DDR. ATM belongs to a conserved family of PI3K-like protein kinases (PIKKs) (Lempiainen and Halazonetis, 2009) that includes, among others, two major DDR transducers: DNA-dependent protein kinase (DNA-PK) (Weterings and Chen, 2007) and ataxia-telangiectasia and Rad3-related (ATR) (Flynn and Zou, 2010). ATM and ATR share substrates in the DSB response but exhibit selective substrate specificities in response to different genotoxic stresses and different DSB inducers (Helt et al., 2005). These three kinases maintain close functional relationships.

2. Chromosomal instability syndrome

In humans, germ-line mutations in genes encoding damage response proteins can lead to inherited "genomic instability syndromes" that involve variable degrees of tissue degeneration (most notably in the nervous and immune systems), sensitivity to specific genotoxic stresses, cancer predisposition, and occasionally premature aging (Negrini et al., 2010; Eyfjord and Bodvarsdottir, 2005; Halazonetis et al., 2008; O'Driscoll and Jeggo, 2006). Knockout of single or combinations of DDR genes in mice leads to phenotypes ranging from neurodegeneration and cancer predisposition (Barzilai et al., 2008; Conmy and Nasheuer, 2010) to premature aging and death in early life (van der Horst et al., 1997; van der Pluijm et al., 2007; Harada et al., 1999; Shiomi et al., 2004, 2005) to embryonic lethality (Ferguson et al., 2000; Gao et al., 2000; Orii et al., 2006). These human and mouse phenotypes attest to the cardinal importance of maintenance of genome integrity in embryonic development and maintenance of tissue homeostasis.

2.1. Ataxia-telangiectasia

ATM mutations that eliminate or inactivate the human ATM protein cause the prototypic genomic instability syndrome, ataxia-telangiectasia (Lavin, 2008; Chun et al., 2004). A-T is an autosomal recessive disease characterized primarily by early onset of progressive neurodegeneration affecting mainly the cerebellum; A-T patients ultimately develop severe, debilitating neuromotor dysfunction. In addition, immunodeficiency that spans the B- and T-cell systems underlies recurrent sinopulmonary infections in some patients. Additional symptoms are thymic and gonadal atrophy, occasional retarded growth, a marked predisposition to lymphoreticular malignancies, and acute sensitivity to ionizing radiation and radiomimetic chemicals. Laboratory hallmarks are chromosomal fragility and clonal translocations that usually herald the onset of lymphoreticular malignancy and high levels of serum alpha-fetoprotein. Cultured A-T cells exhibit severe cellular sensitivity to DSB-inducing agents and have a markedly defective DSB response.

2.2. Nijmegen breakage syndrome

Hypomorphic mutations in the *NBS1* locus lead to the Nijmegen breakage syndrome (NBS), expressed as a combination of microcephaly, mental deficiency, immunodeficiency, radiation sensitivity, chromosomal instability, and cancer predisposition (Digweed et al., 1999; Tauchi et al., 1999; van der Burgt et al., 1996). The *NBS1* gene product, Nbs1, forms a complex with Mre11 and Rad50 (the M/R/N complex), which is involved in sensing and processing DSBs and in maintenance of cell cycle checkpoints (D'Amours and Jackson, 2002). An attempt to elucidate the physiological function of Nbs1 in development failed because of early embryonic death of *Nbs1*-null mice (Dumon-Jones et al., 2003; Zhu et al., 2001). Interestingly, Waltes et al. (2009) have identified and characterized a patient with a RAD50 deficiency that results in a clinical phenotype that can be classified as an NBS-like disorder (NBSLD).

2.3. A-T-like disease

Hypomorphic mutations in the *MRE11* gene lead to another genomic instability disorder: A-T-like disease (A-TLD), which shows marked similarities to A-T. Analysis of three families with affected individuals showed many features of A-T, including the progressive cerebellar degeneration (Stewart et al., 1999; Delia et al., 2004). Although none of the affected individuals exhibited ocular telangiectasia, their clinical presentations were otherwise consistent with the diagnosis of A-T (Stewart et al., 1999). A-TLD is the only A-T-like disease that does not result from *ATM* mutations (reviewed in (Taylor et al., 2004)).

2.4. The interrelations between ATM and the MRN complex

Our recent work has shown that ATM has biological functions that are independent of Nbs1 (Dar et al., 2011). This notion is further supported by the different clinical symptoms of A-T and NBS. Importantly, the human phenotypes corresponding to ATM loss (A-T) and partial loss of Mre11 (A-TLD) are much closer to each other than are A-T and NBS. This phenotypic similarity initially led to the assumption and subsequent observation that Mre11 is required for proper ATM activation specifically, that Mre11s activity was required for this process (Uziel et al., 2003; Dupre et al., 2008). It was also recently suggested that oligonucleotides formed as a result of Mre11-mediated DNA resection as well as single-stranded stretches at the break sites (Shiotani and Zou, 2009) play a role in ATM activation. It appears, therefore, that different components of the MRN complex contribute in different ways to ATM activation and recruitment to damaged sites, with a critical contribution of Mre11s enzymatic activity. This notion is compatible with Shull et al. (2009) who showed that Mre11 and Nbs1 activate different DDR signaling pathways in response to DNA damage.

3. The basic characterization of glial cells

3.1. Astrocytes – the multifunctional cells of the nervous system

The star-shaped astroglial cells make up the majority of glial cells in the brain. These specialized glial cells tile the nervous system and are critically important for the function of the central nervous system (CNS). Since the late nineteenth century, astrocytes have been divided into two main subtypes – protoplasmic and fibrous – on the basis of differences in cellular morphology and anatomical locations (Ramon, 1909). The protoplasmic astrocytes are found throughout the grey matter. These cells exhibit morphology of several stem branches that give rise to many finely branching processes. The fibrous astrocytes are found in the white matter and exhibit a morphology of many long fiber-like processes (Ramon, 1909). In addition to their role in the grey and the white matter it has been shown that protoplasmic and fibrous astrocytes make extensive contracts with blood vessels, and both types of astrocytes form gap junctions between distal processes of neighboring astrocytes (Peters et al., 1991). Golgi (1903) proposed that astrocytes are the cells that metabolically connect neurons with blood vessels. The metabolic support of neurons is achieved through a glucose-lactate shuttle operating within the astroglial