Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Determine the impact of nuclear envelope rupture on cell homeostasis

Synopsis: The nuclear envelope (NE) protects the eukaryotic genome by providing a physical barrier between the chromatin and cytosol. The major components of the NE are the nuclear membranes, which are contiguous with the ER and each other, nuclear pore complexes, which regulate large molecule transport, and the nuclear lamina (NL). The genome contacts the NL in broad lamina-associated domains (LADs), which are believed to aid the spatial organization of chromosomes and contribute to transcription regulation[1].The maintenance of the nucleo-cytoplasmic barrier is essential to protect the chromatin from cytoplasmic enzymes such as nucleases and to prevent unprocessed pre-mRNAs to enter the cytoplasm for translation.
However, the movement of cells or the cell nucleus through confined spaces can cause mechanical stress that results in damage to the nuclear lamina and membrane which may lead to nuclear envelope rupture[2,3]. Our initial observations show in addition, that DNA damage and specifically exacerbated DNA double strand breaks can as well lead to disruption of the balance between the Lamin network and the attached chromatin and results in nuclear membrane blebbing and even rupture. A breach of the nuclear membrane exposes the DNA to cytoplasmatic proteins including DNases such as the three-prime repair exonuclease 1 (TREX1; also known as DNase III). A recent study demonstrated that TREX causes DNA damage, probably by entering the nucleus after nuclear membrane rupture events. The increased DNA damage then triggers downstream pathways which can increase tumor cell invasiveness[4].
Despite the recent progress in understanding the consequences of nuclear envelope rupture events, it is largely unclear how the exchange of molecules between the formerly separated nucleus and cytoplasm takes place and if an immediate damage response mechanism exists. We hypothesize that cells possess acute response mechanisms that act upon nuclear membrane rupture, to i.e., prevent excessive genomic DNA damage by nucleases.
In this project we aim to investigate the events that follow a rupture of the nuclear membrane and measure how the resulting exchange of molecules between the nucleus and cytoplasm takes place. We will use induced DNA damage in cultured cells to effect nuclear envelope rupture and use live cell fluorescence microscopy to determine the distribution and dynamics of molecules in the nucleus and cytoplasm following nuclear membrane breaches.

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