Aberrant interplay between the mutant androgen receptor and GLI3: highlighting a potential neurodevelopmental weakness in Kennedy's Disease

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While the toxic gain of function of the mutant androgen receptor (AR) in Kennedy's Disease (KD) has been widely studied, little is known about the transcriptional dysregulation linked to mutant AR loss of function. To investigate this aspect, we differentiated iPSCs derived from a KD patient (ARQ51) and their isogenic control (ARQ1) into motor neurons (MNs) following a small molecule-based protocol. Among many variations, bulk RNA sequencing revealed *GLI3* downregulation in ARQ51 MNs compared to ARQ1. GLI3 is a key transcriptional regulator of spinal cord and skeletal muscle patterning and/or repair. GLI3 exists in a full-length form (GLI3-FL) behaving as activator and a truncated form (GLI3-R) acting as repressor. The GLI3-FL/GLI3-R ratio is established by Sonic hedgehog and Wnt signaling cascades and spatially regulates tissue specification. Importantly, direct binding and reciprocal regulation between AR and GLI3 have been observed in cancer.
To study the interplay between GLI3 and AR, either wild-type (Q22) or mutant (Q66) AR and GLI3 were overexpressed in motor neuron-like NSC-34 cells and HEK293T cells. In NSC-34 cells, GLI3 was mainly found in the GLI3-R form, while in HEK293T cells GLI3-FL prevailed. In both cell lines, co-expression with ARQ66 did not affect GLI3 protein levels compared to ARQ22. Additionally, both ARQ22 and ARQ66 were shown to bind to GLI3-FL in a testosterone-independent manner. Moreover, GLI3 was partially sequestered within ARQ66 aggregates, suggesting potential consequences on GLI3 proteolytic processing. In NSC-34, ARQ66 potentiated GLI3-R repressor activity, while GLI3-R reduced ARQ66 transcriptional activity to a higher extent compared to ARQ22. In HEK293T cells, ARQ66 resulted less efficient than ARQ22 in enhancing GLI3-FL transcriptional activity. These observations suggest a potentially altered function of mutant AR in the modulation of GLI3-FL and GLI3-R activities.
Together, our data highlight a previously unreported interplay between GLI3 and mutant AR that may have a role during neurodevelopment and in KD pathogenesis.

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