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Treacher Collins syndrome 3 (TCS3)-associated POLR1C mutants are localized in the lysosome and inhibits chondrogenic differentiation

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ABSTRACT

Treacher Collins syndrome (TCS) is a craniofacial developmental disorder whose key feature is a combination of symptoms. For example, a patient could have bilateral downward slanting of the palpebral fissures, colobomas of the lower eyelids, hypoplasia of the facial bones, cleft palate, malformation of the external ears, and atresia of the external auditory canals. TCS3 is caused by mutations of the *polr1c* gene, which encodes RNA polymerase I and III subunit C (POLR1C). There have been two known missense mutations (Arg279-to-Gln [R279Q] and Arg279-to-Trp [R279W]) at the Arg-279 position. However, it remains to be clarified whether or how both or each individual mutation affects the cellular properties of POLR1C. Here we show that TCS3-associated missense mutations cause aberrant intracellular localization of POLR1C, inhibiting chondrogenic differentiation. The wild type POLR1C is normally localized in the nuclei. The R279Q or R279W mutant is primarily found to be localized in the lysosome. Expression of the R279Q or R279W mutant in mouse chondrogenic ATDC5 cells decreases phosphorylation of 4E-BP1 and ribosomal S6 proteins, which belong to the mammalian target of rapamycin (mTOR) signaling involved in critical roles in the lysosome. Furthermore, expression of the R279Q or R279W mutant inhibits chondrogenic differentiation in ATDC5 cells. Taken together, TCS3-associated mutation leads to the localization of POLR1C into the lysosome and inhibits chondrogenic differentiation, possibly explaining a portion of the pathological molecular basis underlying Treacher Collins syndrome.

1. Introduction

Treacher Collins syndrome (TCS) is a craniofacial developmental disorder that features a combination of symptoms [1,2]. In many cases, the patient shows the palpebral fissures and colobomas of the lower eyelids. The symptoms often involve not only defective development of the facial bones and cleft palate but also malformation of the external ears, atresia of the external auditory canals, and hearing loss. TCS affects an estimated 1 in 50,000 people and can be caused by gene mutations. The affected genes are so far known to be three genes of *tcof1*, *polr1c*, and *polr1d*, each of whose mutations causes TCS designated as TCS1, TCS3, and TCS2, respectively [1,2]. Recent advances in sequencing techniques identify gene mutations in some candidate genes associated with TCS and likely increase the number of affected genes.

The functions of these'three TCS affected gene products are well characterized. Treacle ribosome biogenesis factor 1 (TCOF1) interacts with upstream binding factor (UBF) to produce ribosomal RNA [3,4]. RNA polymerase I and III subunit C (POLR1C) and POLR1D are a subunit of both RNA polymerase I (*Pol*I) and III (Pol III) complexes [5,6]. *Pol*I and Pol III produce ribosomal RNA (rRNA). Pol III also participates in synthesizing transfer RNA (tRNA) and other small RNAs. These molecules are all localized in the nucleus. As such, despite well-known cellular functions of these gene products,

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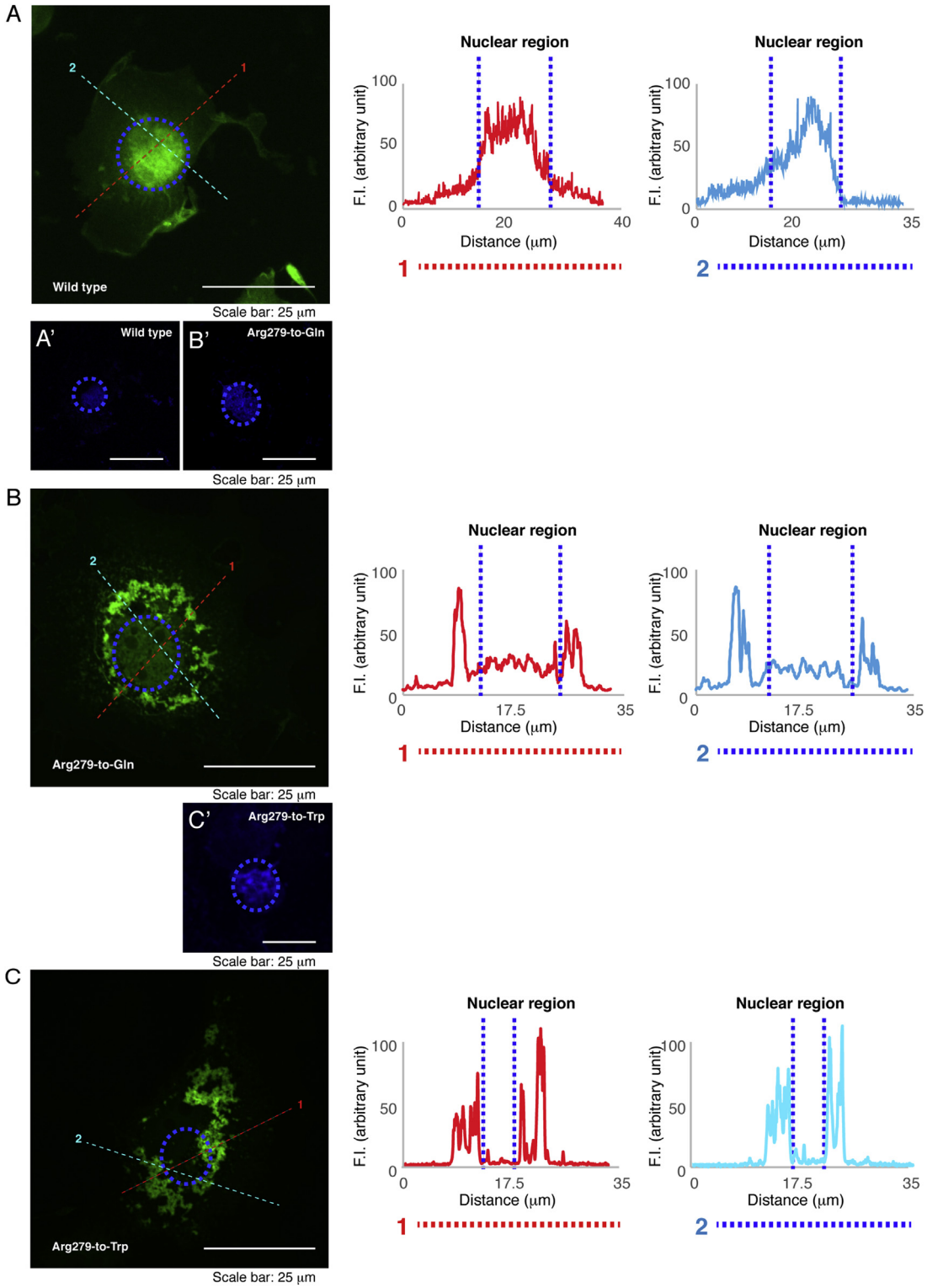

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