Altered Telomeres in Tumors with *ATRX* and *DAXX* Mutations

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A recent study of pancreatic neuroendocrine tumors (PanNETs) revealed that 43% harbored inactivating mutations in the *ATRX* or *DAXX* genes (*1*). The proteins encoded by *ATRX* and *DAXX* interact with one another and play multiple cellular roles, including chromatin remodeling at telomeres, where they are required for the incorporation of the histone variant H3.3 (*2*–*6*). Given the potential role of ATRX and DAXX in modulating telomeric chromatin, we evaluated telomere status in PanNETs in which *ATRX* and *DAXX* mutational status had been determined through Sanger sequencing. Telomere-specific fluorescence in situ hybridization (FISH) revealed that 25 of 41 (61%) PanNETs displayed large, ultrabright telomere FISH signals, a nearly universal feature of the telomerase-independent telomere maintenance mechanism termed alternative lengthening of telomeres (ALT) (Fig. 1) (*7*). *ATRX* and *DAXX* gene mutations both were significantly correlated with ALT positivity ($P < 0.008$ for each gene). All 19 (100%) PanNETs with *ATRX* or *DAXX* gene mutations were ALT-positive (table S1), whereas 6 of 20 cases without detectable mutations were ALT-positive. Subsequent immunolabeling revealed that each of the six ALT tumors lacking point mutations or insertions or deletions had lost nuclear expression of either ATRX or DAXX (Fig. 1, fig. S1, and table S1). In contrast, the 16 tumors without ALT showed robust nuclear labeling for both proteins (table S1), and this relationship was statistically significant ($P = 0.012$ and $P = 0.003$, respectively). Thus, there was a perfect correlation between inactivation of ATRX or DAXX and the ALT phenotype in PanNETs.

To ascertain whether *ATRX* and *DAXX* gene mutations might be more generally associated with the ALT phenotype, we examined 439 tumors of other types. We did not identify any *DAXX* mutations but did identify *ATRX* mutations in cancers of the central nervous system (CNS): pediatric glioblastoma multiforme (GBM) (14.3%), adult GBM (7.1%), oligodendrogliomas (7.7%), and medulloblastomas (1.5%) (Fig. 1 and table S2). To determine whether the ALT status of the CNS tumors was correlated with the presence of *ATRX* mutations, we performed telomere FISH on eight *ATRX* mutant cases in which tumor material was available. In each of these eight cases, extremely bright telomeric foci were identified in the neoplastic cells, and immunolabeling showed loss of nuclear expression of ATRX (Fig. 1 and table S3). We concurrently performed telomere FISH on 16 cases of the same histologic subtypes without detectable mutations of *ATRX* or *DAXX* and found that none had evidence of abnormal telomere foci.

We also studied the human osteosarcoma cell line U-2 OS because this line was a prototype for delineating the ALT phenotype (*8*). We found that exons 2 to 19 of *ATRX* were homozygously deleted in these cells, inactivating the gene product and causing a lack of ATRX immunolabeling (fig. S2).

There is thus a strong correlation between inactivation of *ATRX* or *DAXX* and the ALT phenotype in unrelated tumor types. Previous evidence suggests that the ATRX-DAXX complex functions in heterochromatin assembly at repetitive G-rich regions, such as telomeres (*3*, *5*, *6*). Furthermore, decreasing ATRX or H3.3 in mouse embryonic stem cells results in telomere destabilization and up-regulation of telomere repeat-containing RNA (*6*, *9*–*11*).

Our results are consistent with a model in which loss of ATRX-DAXX function impairs the heterochromatic state of the telomeres, perhaps because of reduced levels of H3.3 incorporation, leading to telomere destabilization and increased HR at the telomeres and thereby facilitating the development of ALT.

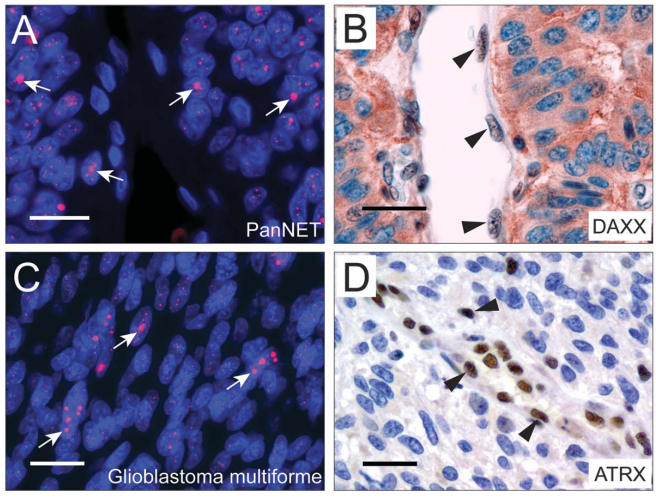

Fig. 1. Representative images of ALT-positive tumors with ATRX or DAXX mutations. (**A**) Example of ALT-positive PanNET. Large, ultrabright telomere FISH signals (red) indicative of ALT are marked (arrows). (**B**) Immunolabeling of the same PanNET shows loss of nuclear DAXX protein in tumor cells. (**C**) Example of ALT-positive GBM. Large, ultrabright telomere FISH signals (red) indicative of ALT are marked (arrows). (**D**) Immunolabeling of the same GBM shows loss of nuclear ATRX protein in tumor cells. In (B) and (D), benign endothelial cells (arrowheads) served as positive immunostaining controls. Scale bars, 30 μm.

References and Notes

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 to S4
References (*12*–*23*)

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