Contribution ID : **19** Type : **not specified**

Human X chromosome reactivation: structural and molecular dynamics during pluripotent reprogramming

lunedì 28 febbraio 2022 14:30 (10)

Reactivation of the inactive X-chromosome (Xi) has been used to model epigenetic reprogramming in the mouse. Human studies have, however, been hampered by Xi epigenetic instability in pluripotent stem cells and difficulties in tracking emerging iPSCs. Recently, we have shown that reprogramming female human fibroblast via mouse ESC fusion recapitulates features of in vivo human naïve pluripotency. We used this unique reprogramming system to examine the earliest chromatin and transcriptional events in Xi reactivation. Our study revealed a rapid (1-2 days) and wide-spread (30-50% of cells) delocalization of XIST RNA and loss of H3K27me3 from the human Xi that precede, and are tightly associated with, the re-expression of selected Xi genes. After cell division, Xi gene reactivation was observed in a similar percentage of hybrids and remained stable over 6 days. The human pluripotency-specific XACT RNA was instead re-expressed and coated the Xi in rare hybrids (1%), suggesting that XACT is not required for early Xi chromatin changes and gene reactivation in the reprogramming context. Collectively, these data distinguish pre- and post- mitotic changes and reveal a hierarchy of epigenetic events that are required for Xi reactivation. Interestingly, single-cell RNA-FISH and allele-specific RNA sequencing analyses showed that reprogramming-mediated human Xi reactivation was partial and selective for a specific subset of genes. Selective Xi reactivation was not limited to gene loci residing within specific chromatin domains neither influenced by proximity to XIST locus. Reactivation was instead associated with stochastic Xi expression ahead of reprogramming, as shown by isogenic fibroblast clones and single cell analyses. Importantly, reprogramming-mediated reactivation remained partial even in cells examined up to six days after fusion, but it was extended to a second group of Xi loci by DNA demethylation. These findings underscore the differential sensitivity of distinct human Xi genes to reprogramming-mediated reactivation and suggest that multiple non-overlapping epigenetic mechanisms maintain silencing along the human Xi.

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