Extracellular and intracellular control of pluripotency: beyond transcription factors

A. Fico, C. D’Aniello, D. De Cesare, E.J. Patriarca, G. Minchiotti

The regulative capability of single cells to give rise to all primary embryonic lineages is designated as pluripotency. Description of fluctuating gene expression and phenotypic heterogeneity in vitro highlighted a conception of pluripotency as an intrinsically metastable and precarious state. However, in the embryo and in defined culture environments the properties of pluripotent cells change in an orderly sequence, moving from the naïve to the primed state, through a cellular continuum. In vivo, blastocyst stem cells progress from a “naïve/ground” state to a “primed” state of pluripotency before lineage commitment.

Such cellular plasticity and its control are crucial in stem cells biology and their application in regenerative medicine. Known molecular markers of stem cell plasticity are mainly transcription factors operating within a pluripotency gene regulatory network. Much less is known on how cellular microenvironment and metabolism regulate this process.

It is now becoming evident that metabolism is a key regulator of stem cell plasticity that has been largely overlooked so far. We have contributed to this emerging field showing that the nonessential amino acid L-Proline acts as an epigenetic signal that drives naïve Embryonic Stem Cells (ESCs) towards an early-primed reversible state of pluripotency (1). Thus, naïve to primed pluripotency transition is regulated by Proline metabolism, we are investigating the underlying mechanism and how it can be extended to human pluripotency.

Pluripotency is largely influenced by microenvironment and extracellular signalling, among which TGFβ pathways exert a key role. We are dissecting the functional role and regulation of the TGFβ superfamily co-receptor Cripto in mouse and human pluripotency. Cripto sustains mouse ESC self-renewal by modulating Wnt/β-catenin, whereas it maintains mouse Epiblast Stem Cells (EpiSCs) and human ESC pluripotency through Nodal/Smad2. Cripto deficiency attenuates ESC lineage restriction in vitro and in vivo, and permits ESC transdifferentiation into trophectoderm lineage, suggesting that it has earlier functions than previously recognized. Our studies contributed to the understanding of the extrinsic regulation of the first cell lineage decision in the embryo (2).

An additional level of control of the stem cell plasticity and pluripotency is represented by noncoding RNAs (ncRNAs). Regulatory ncRNAs come in two flavors: short (miRNAs) and long (lncRNAs). Among the lncRNAs, Transcribed ultraconserved elements (T-UCEs) exert a great interest given their full conservation among human, rat, and mouse genomes, T-UCEs have been mostly studied in cancer, where they can act as “natural sponges” to decoy specific miRNAs. Evidence for the roles of T-UCEs in physiological contexts, such as development and stem cell biology, are currently scarce. We have identified a IncRNA containing the uc.170+, named T-UCstem1, and provided in vitro and in vivo evidence that it plays essential roles in ESCs by modulating cytoplasmic miRNA levels and preserving transcriptional dynamics. In particular, while T-UCstem1::miR-9 cytoplasmic interplay regulates ESC proliferation by reducing miR-9 levels, nuclear T-UCstem1 maintains ESC self-renewal and transcriptional identity by stabilizing Polycomb Repressive Complex 2 on bivalent domains (3, 4). We have recently extended our studies to adult stem cells, and in particular to neural stem cells. By combining gain- and loss-of-function experiments in post-natal mouse brains we demonstrated that T-UCstem1::miR-9 interplay controls neurogenesis by favoring proliferation of neural progenitors at the expense of neuron
production (5). We are now preceding our studies by further analyze T-UCstem1 function in human pluripotent stem cell-derived mini brains and in pathological context such as in Parkinson disease.

References:

Keywords: pluripotency; neural differentiation; non-coding RNAs

Contacts: annalisa.fico@igb.cnr.it; gabriella.minchiotti@igb.cnr.it

Website(s):

Other:
Patent (Nr. 102015000061196) Molecole di RNA isolate e loro usi. Fico A, Cimmino A, Minchiotti G