Cryo nano analysis of changes in the water, dry mass and elements contents during nucleolar stress indiced by different inhibitors of RNA synthesis

Michel, J.[1], Nolin, F.[1], Wortham, L.[1], Tchelidze, P.[1], Banchet, V.[1], Lalun, N.[1], Terryn, C.[1] and Ploton, D.[1]

[1] Université de Reims Champagne Ardenne, France

Some years ago, we developed a new correlative light and cryo-STEM method to quantify water, dry mass and element content (EDX analysis) at a nanoscale resolution within compartments of control, pre-apoptotic and apoptotic cancerous cells (1) (2). Recently, we improved our data analysis to also quantify macromolecular crowding i.e. the percentage of cell volume occupied by hydrated macromolecules.

By using these tools, we here investigated whether different inhibitors of RNA synthesis that induce nucleolar stress provoke similar changes in the molecular crowding and element content within compartments of Hela cells stably expressing H2B-GFP. In this study, we used three RNA synthesis inhibitors: i) CX-5461 that specifically inhibits ribosomal RNA synthesis (rRNA) and induce cell senescence (treatment during 30 h) (3), ii) DRB that inhibits mRNA synthesis and rRNA processing without inducing apoptosis (treatment during 6 h) and iii) Dactinomycin (DAM) at high concentration that inhibits rRNA and mRNA synthesis inducing pre-apoptotic cells (treatment during 5 h). Water, dry mass and elements contents were quantified in five different regions of interest (ROI) of each control and treated cells: condensed chromatin (CC) (identified by the fluorescence of H2B-GFP), nucleolar components (NU), nucleoplasm (NPL), cytosol (CYT) (cytoplasm outside of membrane-limited organelles) and mitochondria (MIT).

In control cells, macromolecular crowding reaches around 50% for CC and MIT, 40% for NU and 30% for NPL and CYT. In cells treated with CX-5461 (senescent cells), molecular crowding increased between 40 to 100% in all cell compartments (80 to 100% for NPL, CYT and MIT).In cells treated with DRB for 6h, molecular crowding increased between 20 to 60% in all cell compartments. At the opposite, in all compartments of cells treated with DAM for 5h (pre-apoptotic cells), molecular crowding decreased by 60% compared to control cells. We then performed a targeted EDX analysis to quantify main elements, N, P, K^+, Na^+, Cl^-, S and Mg^{2+}(calculated in mmol/L) in ROI of main cell compartments. Compared to control cells, we evidenced that treatment with: i) CX-5461 induced an increase of all elements in all cell compartments between 50 to 300%, ii) DRB induced an increase of all elements in all cell compartments between 50 to 150%,iii) DAM induced a decrease of all elements in all cell compartments by 60%.

To complete these results we studied the localization of phosphorylated NF-kB (pNF-kB). Interestingly, we found that pNF-kB was localized within the nucleus (more particularly in the nucleolus) only when cells were treated with DAM.

Altogether, this study uncovers that several RNA synthesis inhibitors induce strong different changes in cellular biophysical properties such as molecular crowding and element contents. We hypothesize that these changes have important effects on cell metabolism (4) and on inhibition or activation of some regulatory proteins such as NF-kB.

(1) Nolin F. et al. Cell Mol Life Sci (2013), 70: 2383-2394
(2) Nolin F. et al. Plos One (2016), 11: e0148727
(3) Drygin D. et al. Cancer Res. (2011), 71 : 1418-1430
(4) Mourao M. et al. Biophys. J. (2014), 107 :2761-2766