Cell guidance into quiescent state through chromatin remodelling induced by elastic modulus of substrate

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Statement of Purpose: Substrate stiffness is known to strongly influence the fate of adhering cells. Yet, little is known about the influence of the substrate stiffness on chromatin. Chromatin integrates a multitude of biochemical signals interpreted by activation or gene silencing. We investigate the impact of substrate elasticity on nuclear components, which led us to demonstrate that the remodelling between euchromatine and heterochromatin, together with the nuclear envelope connected to intermediate filament (IF) network, are major determinants of the response of epithelial cells to external mechanical signals.

Methods: We used marsupial kidney epithelial (Ptk2) cells deposited on polyelectrolyte multilayers (PEMs). The PEM mimic microenvironments of various elastic moduli, composed of a hyaluronic acid/poly-L-lysine $(PLL/HA)_{24}$ stratum capped by a second poly(styrene) sulfonate/polyallylamine $(PSS/PAH)_n$ stratum (n=0, 1, 2 and 5) with Young modulus ranging from 200 down to 0 kPa (Kosgozlu et al., 2010; Rabineau et al., 2013).

Results: We report that on stiff substrates (100-200 kPa), where cells preferentially adhere, chromatin is mainly found in its euchromatine form. Decreasing the Young modulus to 50 kPa (E_{50}) is correlated with cell rounding and with a partial shift from euchromatin to heretochromatin. These cells are still surviving without detection of apoptotic and necrotic markers. On very soft substrates (<<10 kPa, E_0), for the majority of cells this is accompanied by cell lysis, resulting in euchromatine and heterochromatin protein 1 β (HP1β) released from nuclei and ultimately to cell death by necrosis (Fig. 1 E_0).

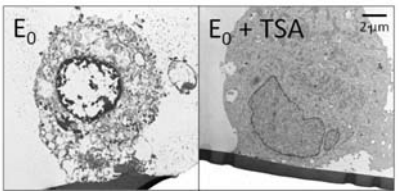

Figure 1. Ultrastructure of PtK2 cells on very soft substrate (<<10 kPa) with or without TSA, by electronic microscopy.

Highlighting, on these very soft substrates, histone deacetylase inhibition by adding a drug (trichostatin A: TSA) preserves actetylated histone and thus maintains the euchromatine form (Fig. 1 E_0+TSA and Fig. 2) with uniform distribution of HP1β in the nucleus, thereby keeping intact the nuclear envelope as well as a residual intermediate filament network around the nucleus. This allows cells to survive in a non-adherent state without undergoing proliferation (Kocgozlu et al., 2012) and independently of their transcriptional competence.

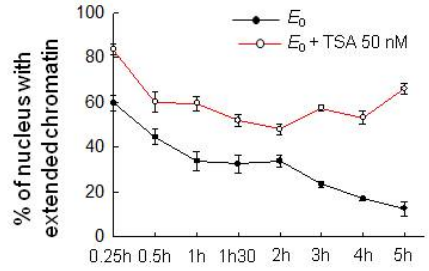

Figure 2. *Percentage of cells with decondensed nucleus during different time-periods after cell speeding on E_0 with or without TSA.*

When transfer on a stiff substrate these cells retain their capacity to adhere, to spread and to enter a novel mitotic cycle in a way that depends on their transcriptional competence (Fig. 3A). A similar effect is observed on soft substrates (50 kPa) without need of histone deacetylase inhibition (Fig. 3B). These new results suggest that on soft substrates cells might enter in a quiescence state (Rabineau et al., 2014).

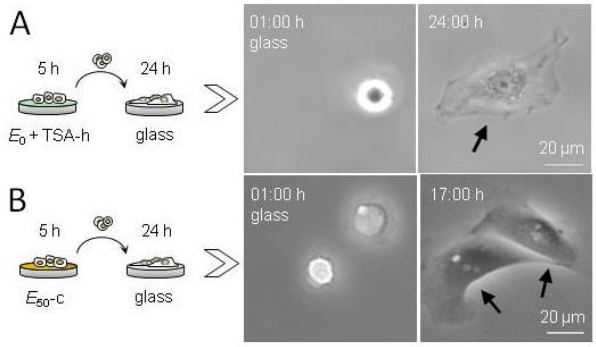

Figure 3. PtK2 cells seeded either on E_0+TSA or on E_{50} spreading after transfer on glass, by videomicroscopy.

Conclusions: This work, has reveals an unexpected relationship, between substrate elasticity and chromatin plasticity in epithelial cells. Together, these results demonstrate that the nucleus represents a level of integration of mechanical inputs. Its response to external signals provided by soft substrates depends on a residual IF network connected to the nuclear lamina. These findings might be relevant to maintain cells in the best settings within synthetic scaffolds and in tissue-derived matrices used in tissue regeneration strategies.

References: Kocgozlu et al., J Cell Sci, 2010, 123 (1) : 29-39. Kocgozlu et al., Biomaterials, 2012, 33 :798-809 ; Rabineau et al., PloS ONE, 2013, 8(10) : e78468. Rabineau et al., Biomaterials, 2014, 10.1016/j.biomaterials.2014.10.023.