ImmunoTools *special* Award 2014

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Characterisation of Secretory Phenotype of Human Dermal Fibroblasts upon ageing of the nuclear lamina.

The nuclear lamina is a vast protein meshwork that lies beneath the inner membrane of the nuclear envelope. Research from many laboratories, including ours, has demonstrated that the lamins are involved in a wide range of functions, including maintenance of nuclear integrity, regulation of DNA replication, control of gene expression and epigenetic state of chromatin. Therefore it is not surprising that mutations of lamina components may lead to many different human diseases referred to as the laminopathies. One of them is Hutchinson-Gilford progeria syndrome (HGPS), regarded by many as a premature ageing syndrome. HGPS is associated with a mutation in the *lamin A* gene (LMNA), which causes aberrant processing of prelamin A. This mutation results in production of a truncated form of lamin A (missing 50 internal amino acids), called progerin, which retains farnesylation at the C-terminus, a posttranslational modification normally removed from wild type lamin A. Accumulation of farnesylated progerin has been shown to be a major factor inducing senescence and is associated with profound changes to the nuclear lamina, with a wide range of functional consequences important to the development of this progressive ageing syndrome. Some of these pathological lamina changes, including accumulation of farnesylated prelamin A are now being found in normal aged tissue.

Based on these findings, we have established two cell models of premature ageing, pharmacological and genetic. Both of them involve perturbation of normal processing of lamin A in a way that results in accumulation of farnesylated prelamin A, thus, mimicking the phenotype observed in HGPS, at least at cellular level. In the pharmacological model we use a drug called Saquinavir that inhibits Zmpste24, a proteinase responsible for maturation of prelamin A and removal of the farnesylated C-terminus. The genetic approach involves knocking-down of Zmpste24 with siRNA and, similarly, leads to accumulation of farnesylated prelamin A. Both models have been optimised and we demonstrated that cells depleted of functional Zmpste24 not only accumulate farnesylated prelamin A, but also undergo senescence. Their morphology changes, they undergo proliferation arrest and exhibit a number of senescence markers, like increased expression of $p16^{INK4A}$ or senescence associated β-Galactosidase activity. To complement these two models we also included a physiological model that involves passaging of early passage primary human dermal fibroblasts (young HDFs) until they reached cell cycle arrest and cellular senescence (becoming old HDFs).

Although senescent cells cannot proliferate anymore, they remain metabolically active and secrete a wide range of soluble and insoluble proteins, including interleukins, chemokines, proteases, growth factors and components of ECM. Collectively they are called the senescence associated secretory phenotype (SASP) and are a hallmark of senescence. SASP is believed to induce chronic inflammation which affects not only neighbouring cells but can also become widespread. We have performed a genome-wide microarray analysis of transcriptome in our ageing models that yielded a substantial number of candidates potentially involved in regulation of ageing linked to the nuclear lamina, among them are many cytokines. Now we are in the process of collecting data at the protein level and characterising SASP in our ageing models. ImmunoTools would be very useful to asses levels of specific interleukins that have been shown to play a key role in regulation of senescence (e.g. IL-6, IL-8), but we need to further explore differences between SASP in physiological ageing and the one induced by nuclear lamina abnormalities. Our results will provide a new insight into links between nuclear envelope and immunoresponse of aged cells and the ImmunoTools will really help in this investigation and validation of the role of specific cytokines in this process.

ImmunoTools *special* AWARD for **Marek Drozdz** includes 25 reagents

recombinant human cytokines rh EGF, rh FGF-b / FGF-2, rh G-CSF, rh GM-CSF, rh GRO-alpha, rh HGF, rh IL-1alpha / IL-1F1, rh IL-1beta /IL-1F2, rh IL-6, rh IL-7, rh IL-8, rh IL-15, rh IL-17A, rh MCP1 / CCL2, rh MCP2 / CCL8, rh MCP3 / CCL7, rh MIP-1α/ CCL3, rh RANTES / CCL5, rh SCF, rh SDF-1α / CXCL12a, rh TNFα, rh TRAIL / CD253, rh VEGF-A/VEGF-165

human IL-6 ELISA-set, human IL-8 ELISA-set (each 3 reagents)

DETAILS