Stem Cells Cycle Towards Immune Surveillance

Ashleigh S. Boyd[1, 2] and Neil P. Rodrigues[3]

[1]Department of Nanotechnology, UCL Division of Surgery and Interventional Science, University College London, Royal Free Hospital, London, NW3 2PF, England, United Kingdom.

[2]UCL Institute for Immunity & Transplantation, University College London, Royal Free Hospital, London, NW3 2QG, England, United Kingdom

[3]The European Cancer Stem Cell Research Institute, Cardiff University School of Biosciences, Cardiff, CF24 4HQ, Wales, United Kingdom.

Correspondence:
a.boyd@ucl.ac.uk
RodriguesN@cardiff.ac.uk

Immune surveillance is an established regulatory mechanism that spares tissues from malignant transformation. Agudo et al. find that the chief cell type to generate tissues in the body – somatic stem cells - is only subject to immune surveillance during proliferation.

Somatic stem cells (hereafter stem cells) are tasked with the substantial responsibility of managing and sustaining the functionality of individual tissues. Stem cells remain relatively quiescent until times of physiologic need when they are fated to self-renew and differentiate into more specialized cells that comprise a tissue. Regulation of quiescence (as well as self-renewal and differentiation) has been conjectured to be of importance in conservation of the usually rare stem cell pool and preventing genetic/epigenetic mutations, which could ultimately generate cancer. To this end, cell cycle inhibitors and attendant DNA damage repair pathways have been identified as crucial regulators of stem cell homeostasis (Cheng et al., 2000). If mutations remain unchecked in differentiated cells, additional mechanisms such as immune surveillance mechanisms come to the fore to remove mutated cells. For example, mutations may up-regulate immunogenic ligands, and cytokines and chemokine production that trigger an innate immune response mediated by NK cells. Whether stem cells, like their differentiated progeny, are monitored by the immune system remains an unanswered question that is addressed in the current study by Agudo and colleagues.

Making elegant use of the Jedi T cell mouse model, in which CD8^{+} **J**ust **e**nhanced green fluorescent protein (EGFP) **d**eath-**i**nducing (Jedi) T cells recognise GFP as their cognate antigen, the authors asked whether epithelial stem cells in multiple tissues were subject to immune surveillance. Jedi T-cells, when transferred into EGFP reporter mice, systematically deplete EGFP-expressing cells and enable examination of T cell responses toward defined cell populations. By adoptive transfer of Jedi T cells into Lgr5-GFP reporter mice, that label epithelial stem cells of the intestine, ovary, mammary gland and hair follicle stem cells (HFSCs), and subsequent vaccination with GFP to activate a T-cell response, the authors found Jedi-T cell treated hosts, but not those receiving control T cells, were depleted of stem cells in each of those tissues apart from HFSCs. Notably, rather than killing HFSCs, transferred Jedi T cells simply surrounded the HFSCs in their niche, leaving them intact. Closer scrutiny of the apparent immune privilege of HFSCs revealed that resistance to T-cell mediated clearance of HFSCs applies only to the resting (telogen) phase of growth, since when Jedi T cells were transferred to Lgr5-GFP hosts during the active (anagen) phase of hair growth they were once again able to deplete Lgr5^{+} HFSCs. Lgr5^{+} HFSCs during the telogen phase were largely devoid of MHC-I expression and β2-microglobulin (β2-m) expression, which is required for proper MHC-I complex formation and display at the cell surface, suggesting perturbed antigen-presentation capacity. Linking this observation to cell cycle

status, it was shown that down-regulated MHC-I expression is a specific feature of non-proliferating HFSCs. In striking contrast, epithelial stem cells from the gut and ovary, which proliferate more vigorously, all expressed MHC-I. The authors reveal that Lgr5^{+} HFSCs in the telogen phase express lows levels of cell cycle genes, consistent with quiescence, and attenuated expression of MHC-I and antigen presentation genes, including NLRC5, a transcriptional regulator of MHC-I and associated genes. The authors extend their work and reproduce their data in another population of slow cycling stem cells, satellite cells. Thus, overall, the data suggest a model whereby 'slow' cycling stem cells are able to bypass immune surveillance and destruction in association with low MHC-I expression and defective antigen presentation, whereas, 'fast' cycling stem cells retain normal MHC-I expression, inducing T cell activation and cytotoxicity (**Figure 1**).

The proliferative status of the epithelial stem cell types studied here is variegated. For example, quiescent mammary stem cells have been isolated within the Lgr5^{+} fraction (Lloyd-Lewis et al., 2017), and, as the authors note, 'reserve' stem cells, with lower proliferative capacity, have been identified within the intestine. Therefore, mammary and intestinal stem cells should not be considered as entirely proliferative stem cell populations. Rather, within those tissues immune surveillance will differ depending on the proportion of stem cells that are proliferating. Further experiments should attempt to discern the impact of quiescence on immune surveillance in each of these classes of stem cells. Levels of interaction between the stem cell and the immune system are also variegated in this study. There is a strong association between intestinal stem cells and the immune system due to the presence of gut-associated lymphoid tissue (GALT), an important site of tolerance induction; on the contrary ovary stem cells are likely to be more immune privileged. These caveats notwithstanding, Agudo et al. provide evidence that stem cell quiescence, rather than the level of interaction of the stem cell with the immune system or other cell-intrinsic property of a stem cell, is a crucial determinant of immune surveillance capacity.

So why are non-proliferating stem cells less susceptible to immune surveillance than their cycling counterparts? As a cardinal aspect of their regulation, quiescence could be part of an evolutionary constraint imposed upon relatively rare stem cells, preventing their premature exhaustion. Yet paradoxically an increasingly appreciated conundrum in the field, and also implied by the work of Agudo et al, is that DNA damage to quiescent stem cells may result in accrual of mutations that escape immune surveillance (as well as DNA repair mechanisms) (Rossi et al., 2007). Further indirect evidence for this hypothesis is supported by the work of Shlush and colleagues (Shlush et al., 2014) who found that in adult patients with a type of blood cancer, acute myeloid leukaemia (AML), a pre-leukaemic 'founder' mutation that originated in haematopoietic stem cells (HSCs) was still observed during remission following chemotherapy. Given that standard chemotherapy in AML targets cycling cells, this suggests a proportion of pre-malignant HSCs are quiescent and capable of escaping chemotherapy-mediated killing. The scene is now set for investigations to determine precisely how quiescent, pre-malignant stem cells, such as those observed in AML, can circumvent an incipient innate (NK mediated) immune response, as exemplified by quiescent HFSCs. Absence of MHC-I should be detrimental to quiescent stem cells as natural killer (NK) cells survey for MHC-I in order to identify and purge transformed cells. Why these non-proliferating stem cells evade NK cells is unclear, but it is possible they may express inhibitory accessory receptors/molecules that signal to NK cells to block its cytotoxic response. Efforts to understand the effectiveness of the known cross-talk between the innate response and the later (T-cell mediated) adaptive immune response in the pre-malignant stem cell setting are also warranted.

Pre-malignant cells, on stepwise acquisition of further mutations in either stem cells or their more differentiated progeny, transform into cancer stem cells (CSCs) – the driving force underpinning clonal growth of cancer and disease relapse in multiple tissue settings (Kreso and Dick, 2014). Recent studies have suggested that specific permutation and combination of mutations acquired in the course of cancer and CSC development may be a crucial determinant of clonal evolution (or outgrowth) of the cancer and clinical outcome (Ortmann et al., 2015; Shlush et al., 2014). Using Ockham's razor, an implication of the current study is that secondary mutations that induce a proliferative state may be effectively controlled by the immune system, checking pre-malignant stem cell growth and CSC generation, whereas those secondary mutations that induce quiescence or a similar state via differentiation arrest may conceal antigenic stimuli recognizable by immune surveillance. Cellular context of mutational acquisition is also of salience in consideration of CSC clonal generation or evolution, with mutations arising within a more proliferative cellular compartment likely to be more prone to effective immune control than those emanating from a relatively quiescent one. As alluded to above, this may impose a selective pressure on the survival of a relatively quiescent pre-malignant stem cell clone which ultimately facilitates the development of aggressive CSC clonal evolution and poor clinical outlook. This hypothesis remains to be tested. Another consideration is that CSCs, like their normal stem cell counterparts, reside in a complex niche that can either (re)-enforce CSC generation/survival or perturb its behavior. In either setting, the correlation between CSC proliferative status, niche biology and immune surveillance should be explored. Extending the observations of this study to define the transcriptional program and function of NLRC5 in quiescent versus cycling CSCs may provide opportunities to therapeutically enhance immune surveillance of CSCs.

In the context of the normal ageing of tissues, where malignancy is kept at bay, immune surveillance still wanes but the impact of this on the ancestral stem cell remains an underexplored area of research. Stem cells typically lose their quiescent status during ageing, which may lead to either an enhanced state of cellular senescence (observed in satellite stem cells) or, alternatively, enhanced proliferation (observed in HSCs) (Artandi et al., 2015). While it is known that cellular senescence can be cleared by antigen-specific immune responses in some tissues (Kang et al., 2011), it will also be of considerable interest to interrogate immune surveillance in tissues where enhanced stem cell proliferation is observed during ageing (e.g. HSCs). The data from Agudo et al. predict that during ageing residual immune surveillance could eradicate the vast majority of mutated cells in the setting of enhanced proliferation and senescence; admittedly its effectiveness in these contexts is also likely to also be contingent on the complex interplay and extent of DNA damage repair deregulation and epigenetic deregulation, which, in the case of Dnmt3a, can confer a self-renewal advantage on stem cells (Shlush et al., 2014).

This sophisticated paper by Agudo et al. lays the foundation for further investigation into the molecular mechanisms associated with immune surveillance in stem cell populations. Continuing to understand why quiescent stem cells are resistant to immune surveillance while their cycling counterparts are not will be of importance in regenerative medicine, where stem cell therapeutics is envisaged. Harnessing that knowledge could also be of utility in immune surveillance based therapies and other components of the clinical armamentarium in cancers that are predominantly driven by cancer stem cells.

References

Agudo, E.S. Park, S.A. Rose, E. Alibo, R. Sweeney, M. Dhainaut, K.S. Kobayashi, R. Sachidanandam, A. Baccarini, M. Merad, B.D. Brown (2018). Quiescent tissue stem cells evade immune surveillance. Immunity, 48 (2), pp. 271-285

Artandi, S.E., Blau, H.M., de Haan, G., Geiger, H., Goodell, M.A., Jones, L., Levine, R.L., Munoz-Canoves, P., Rodewald, H.R., Wagers, A*., et al.* (2015). Stem Cells and Aging: What's Next? Cell stem cell *16*, 578-581.

Cheng, T., Rodrigues, N., Shen, H., Yang, Y., Dombkowski, D., Sykes, M., and Scadden, D.T. (2000). Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science *287*, 1804-1808.

Kang, T.W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., Hohmeyer, A., Gereke, M., Rudalska, R., Potapova, A*., et al.* (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature *479*, 547-551.

Kreso, A., and Dick, J.E. (2014). Evolution of the cancer stem cell model. Cell stem cell *14*, 275-291.

Lloyd-Lewis, B., Harris, O.B., Watson, C.J., and Davis, F.M. (2017). Mammary Stem Cells: Premise, Properties, and Perspectives. Trends Cell Biol *27*, 556-567.

Ortmann, C.A., Kent, D.G., Nangalia, J., Silber, Y., Wedge, D.C., Grinfeld, J., Baxter, E.J., Massie, C.E., Papaemmanuil, E., Menon, S*., et al.* (2015). Effect of mutation order on myeloproliferative neoplasms. N Engl J Med *372*, 601-612.

Rossi, D.J., Seita, J., Czechowicz, A., Bhattacharya, D., Bryder, D., and Weissman, I.L. (2007). Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. Cell Cycle *6*, 2371-2376.

Shlush, L.I., Zandi, S., Mitchell, A., Chen, W.C., Brandwein, J.M., Gupta, V., Kennedy, J.A., Schimmer, A.D., Schuh, A.C., Yee, K.W*., et al.* (2014). Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature *506*, 328-333.