**Multiplicity of stem cell memories of inflammation and tissue repair in epithelia**

**Multiplicity of stem cell adaptive programs to inflammation and tissue damage in epithelia**

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Abbreviations:
**IFE**: interfollicular epidermis; **SCs**: stem cells; **HF**: hair follicle; **SCCs**: squamous cell carcinoma.

## Abstract
Cell adaptation enables cells to change their behavior in response to stimuli. While adaptive programs of immune cells have been widely described, it has recently emerged that also epithelial cells *in vivo* acquire memories. Here we discuss the adaptations identified in epithelia and describe the associated long-term consequences, while proposing their classification.

## Epithelial stem cells face inflammation and injuries
Epithelia form the interface with the environment, lining the body cavities. They act as a protection against pathogens, mechanical and chemical assaults, and have additional absorptive or secretory roles[1]. Thus, they behave as barriers, that constantly sense, respond and adapt to external and internal signals that they routinely encounter[2,3]. Notably, within the several cellular compartments in epithelia, the SCs exhibit remarkable plasticity in response to damage, inflammation and cancer, emphasising their special role in adapting to stimuli and stressors[1,3].

## Spectra of adaptive programs in epithelia
Exceptionally diverse adaptive programs have been described for innate immune cells. Several such memories have been defined, including differentiation, priming, tolerance and trained immunity[4]. Emerging evidence highlights the existence of such a variety of adaptations in epithelial cells, following both inflammation and tissue damage. Based on the classification of innate immune responses[4], we describe and categorise the memories of epithelial cells that have been recently published (**Table 1** and **Figure 1A**, **B**).

- Trained immunity-like adaptation

Upon exposure to inflammatory stimuli, innate immune cells mount a faster and greater response against a secondary challenge. This trained immunity occurs through the persistence of epigenetic alterations even after the stimulus ceases[4]. Back in 2017, it was reported that epidermal SCs in the skin adapt as well to inflammation (chemical or pathogen-driven) or injury, opting for a trained immunity-like response[5]. Indeed, one month after the stimulus, the IFE SCs look transcriptionally similar to naïve cells (untrained), while carrying an epigenetic memory that relies on the inflammasome activation. Thus, the memory of a previous inflammatory event is not anymore exclusive to immune cells[5]. The same trained immunity-like memory has been described for HF-derived IFE SCs and lower-HF SCs in the context of tissue damage[6,7]. Differently from inflammation, mechanical injury triggers an epigenetic memory of migration-related genes[6,7]. In the intestine, upon maternally restricted pathogen-infections, IL-6 exposure mediates a tissue-restricted effect on fetal intestinal epithelium. The global increase of chromatin accessibility with minor (only 6 genes significantly upregulated) impact on their transcriptional landscape characterizes the trained immunity-like adaptation of these intestinal epithelial cells[8].

- Priming

In priming, the initial stimulus induces a change in the functional state of the cell and the event-activated transcription does not return to naïve levels when the stimulus ceases[4]. Recently, specific epidermal cells have been described to undergo priming, therefore acquiring a memory of tissue repair[7]. Indeed, after the complete resolution of an injury, Lrig1$^{+}$ SCs residing in the upper-HF remain transcriptionally pre-activated. This is supported by chromatin rearrangements in genes associated to metabolism and migration, acquired after the injury[7]. However, priming is not limited to the skin. In the pancreas, upon recovery from a transient inflammation, epithelial cells acquire inflammatory memory displaying an enduring adaptive response associated with sustained transcriptional and epigenetic reprogramming[9,10]. After a resolved pancreatitis acinar cells fail to return to their transcriptional baseline even after several months, due to the establishment of an epigenetic memory[9,10]. Additionally, a priming-like phenomenon has been described for intestinal SCs following allotransplantation[11].

- Differentiation and memory niche origin

Innate immune cell 'differentiation' refers to the change of a naïve cell into its mature counterpart, which is defined by a long-term change in the functional program of the cell. This process is usually accompanied by altered morphology caused by alterations of the tissue environment or chronic exposure to stimuli[4]. It is complex to translate the immune differentiation memory concept to epithelia that are already composed in homeostasis by cells with different grades of differentiation and multiple epithelial lineages[1]. However, it seems reasonable to categorise the adaptation process recently described for the human upper airway epithelium, as differentiation-like memory[12]. The analysis of the transcriptional and chromatin profile of chronic rhinosinusitis patients revealed marked differences between non-polyp and polyp epithelial cells. Polyps display a reduction in cellular diversity, with a depletion in glandular and ciliated cells, and an enrichment in basal cells that are shifted towards a secretory (antimicrobial) function[12]. Additionally, after skin injury, the SCs that leave their HF niche to heal the wound, change their lineage towards an IFE identity[7], while maintaining a chromatin memory of their HF origin[6]. Therefore, epithelial cells exhibit additional adaptive spectrum that represent differentiation and the memory of their niche origin.

## Spatial distribution of non-immune cells' memory

Epithelial SCs receive cues from their local microenvironment such as infiltrating immune cells, circulating factors and mechanical forces that direct inflammatory responses and wound repair[5]. Since these secondary effectors are known to have spatially wide effect, the spatial extent of memory is a crucial aspect that has only recently begun to be deciphered. In the case of inflammatory memory caused by the treatment of skin with chemicals, epidermal memory seems locally restricted to the inflamed areas[5]. On the other end, a localized tissue damage is able to elicit an unexpected spatially wide response in epidermal cells, named *distal memory*, with a tissue-scale impact on the skin[7]. The spatial effect of an injury on other epithelia still needs to be elucidated.

## Long-term consequences

In immune cells, over time, these adaptations shape an individual identity, increasing the fitness of organisms against pathogens. Besides the positive effect, adaptations can be maladaptive and predispose to irreversible pathologies[4,13]. Thus, the study of the durability and the reversibility of memory in immune e non-immune cells is crucial to understand the physiological to pathological transition. Epithelial SCs, due to their longevity, display in general long-lasting memories (**Table 1**). In skin, SCs with trained immunity-like adaptation have been observed until 180 days after inflammation[5], while primed SCs last even longer, 40 weeks after skin injury[7]. Similar timing has been described in pancreas, where memory epithelial cells retain chromatin and transcriptional activation till 18 weeks after inflammation[10], and intestine, where memory lasts for 6 weeks, at least[8]. Those long-lasting memories are beneficial in terms of protective action from subsequent inflammation or damage[5–8,10,11] (**Table 1** and **Figure 1C**). However, prolonged chronic exposure to those stimuli reverts the benign effects of memory into detrimental. Priming is crucial to protect pancreas from damage but push the malignant transformation in presence of oncogenic KRAS[9,10]; in intestine trained immunity-like mechanism offers protection from subsequent gut infections but mediates an increased inflammation in the context of colitis[8]; in skin, although cell priming increases tissue repairing abilities, it promotes SCCs onset in UV-exposed epidermis, establishing an epigenetic field cancerization[7].

## Concluding remarks

Overall, the epithelial community just started to understand memory adaptive spectra in SCs, therefore their classification on the basis of their transcriptional and epigenetic features remain limited. In immunology, the opposite of trained immunity is immune 'tolerance,' wherein the cell does not perform its functions following restimulation. Although it has not been described so far in epithelia, a tolerance-like adaptive program might be opted by epithelial cells that encounter specific assaults.

Additionally, due to the heterogeneity of bulk and single cell omics used, it is often not obvious to discriminate between the multiplicity of SCs adaptive programs to inflammation and tissue damage. Anyway here, we might have laid the foundation for further discussion.

Here we summarized the adaptive programs described in 'normal' non-tumor cells that form epithelia. However, similar memory-like mechanisms have been described in tumor cells, during their acquisition of drug-resistance[14], thus suggesting that epithelial memory and its associated molecular mechanisms are highly translationally relevant. This further highlights the importance of deeper knowledge on the memory in adult epithelial SCs.

| | Epithelia | | | Memory | | | Consequences | |
|---|---|---|---|---|---|---|---|---|
| **Reference** | **Organ** | **Cell Type** | **Stimuli** | **Type** | **Spatial extent** | **Duration** | **Beneficial** | **Maladaptive** |
| Naik S. et al., 2017 | skin | IFE SCs | fungi, TPA, wound, imiquimod | Trained immunity-like | limited to the inflamed area | 180 days | enhanced response to repeated challenge | na |
| Gonzales K.A.U. et al., 2021 | skin | low-HF SCs | injury | Trained immunity-like, Memory of niche | na | 80 days | enhanced response to repeated challenge | na |
| Lim A.I. et al., 2021 | intestine | intestinal SCs | maternal infection with Y. pseudotuberculosis | Trained immunity-like | na | 8 weeks | enhanced protective immunity from infections | increased inflammation in the context of colitis |
| Levra Levron C. et al., 2023 | skin | low-HF SCs, up-HF SCs | injury | Trained immunity-like, priming | damage proximity, 7 mm from damage | 8 weeks, 40 weeks | enhanced response to repeated challenge | predisposition to SCCs onset |
| Del Poggetto E. et al., 2021 | pancreas | acinar cells | caerulein | priming | na | 3 months | limited tissue damage after following assault | cooperation with oncogenic Kras to induce tumors |
| Falvo D.J. et al., 2022 | pancreas | acinar cells | caerulein | priming | na | 18 weeks | diminished metaplasia in response to a second insult | increased tumorigenesis with an oncogenic Kras mutation |
| Reddy P. et al., 2023 | intestine | intestinal SCs | gastro-intestinal acute graft | priming | na | 7 days | na | na |
| Ordovas-Montanes J. et al., 2018 | lung | basal SCs | allergy | differentiation-like | na | na | na | contribution to the persistence of human chronic rhinitis |

**Table 1**. Current literature on adaptive programs (memories) in epithelial cells after inflammation or tissue damage. na, not applicable; TPA, 12-O-tetradecanoylphorbol 13-acetate.

## Figure Legends

**Figure 1**. **Spectra of adaptive programs opted by epithelial cells after inflammation or tissue damage and their consequences**. (**A**) Trained immunity-like adaptation, Priming, Differentiation and Memory of niche origin. (**B**) Transcriptional difference between Priming and Trained immunity-like adaptation. (**C**) The epigenetic landscape Waddington's epigenetic landscape describing that the presence of a previous memory epigenetically predisposes cells to tumour formation. The coloured spheres represent different epithelial cell states: (red) cells in homeostasis; (orange) memory cells state, acquired as an adaptation to inflammation/ injury; (yellow, green) cells activated to solve the first (yellow) or the second (green) assault; (violet) neoplastic cell.


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## Declaration of interests

The authors declare no competing interests.

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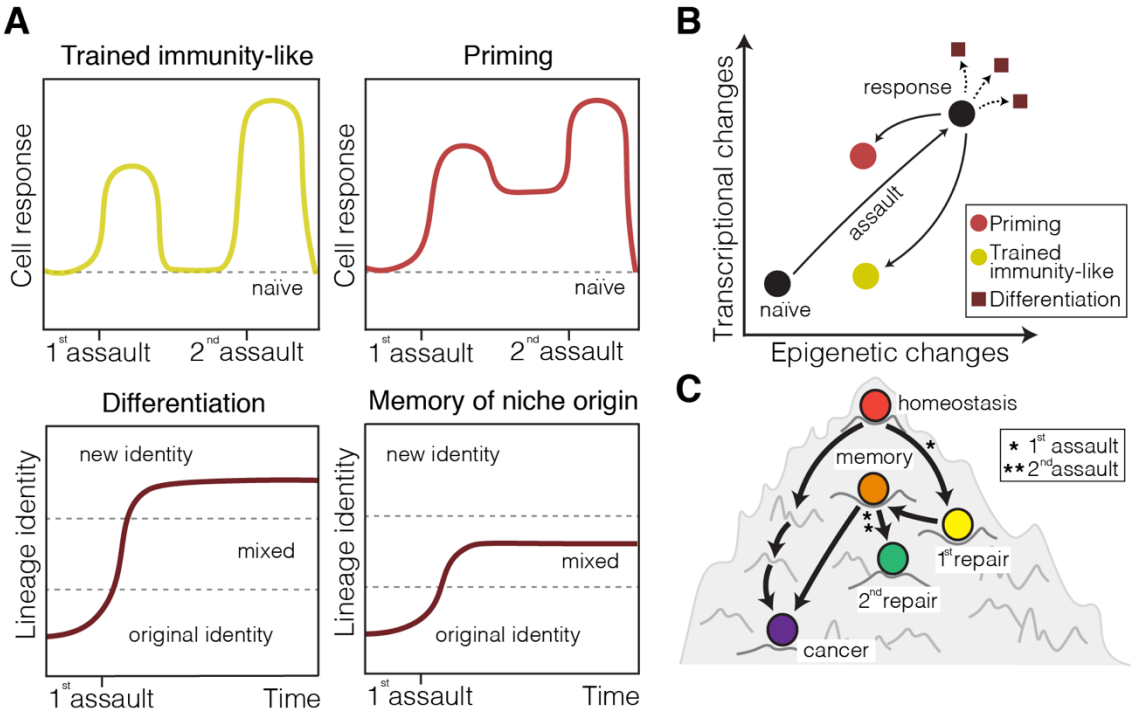
A
Trained immunity-like
Priming
Cell response
naive
1st assault
2nd assault
Differentiation
Memory of niche origin
Lineage identity
new identity
mixed
original identity
Time
B
Transcriptional changes
Epigenetic changes
response
assault
naïve
Priming
Trained immunity-like
Differentiation
C
homeostasis
memory
* 1st assault
** 2nd assault
1st repair
2nd repair
cancer