this close proximity were not fully understood previously.

Lai *et al*. assessed the role of nociceptors in the gut of mice infected with the pathogen *Salmonella enterica* serovar Typhimurium. The authors report that the presence of a subset of gut nociceptors (specifically, those that express the ion-channel proteins TRPV1 and $Na_V1.8$) protect the gut against invasion by *Salmonella* and the subsequent spread of this bacterium to sites such as the liver and spleen. Intriguingly, the authors found that the protective effects of nociceptors were not mediated by well-known antimicrobial defence mechanisms, such as activation of immune cells or alterations in the levels of antimicrobial peptides that are produced by gut cells. Instead, during infection with *Salmonella*, these nociceptors orchestrated a reduction in the number of M cells. Because M cells are a key entry point for *Salmonella*, this reduction would probably have the consequence of reducing the surface area available for *Salmonella* to invade.

The authors analysed the composition of gut bacteria in the absence of *Salmonella* infection, using mice with gut nociceptors that were genetically engineered to lack either TRPV1 or $Na_V1.8$ channel proteins. Compared with animals that expressed these proteins, both types of engineered mouse had lower levels of segmented filamentous bacteria (SFB), a group of commensal microbes that attach to gut epithelial cells, and particularly to M cells[12]. Such commensal bacteria are crucial for providing resistance against gut colonization by pathogens, including *Salmonella*[13].

Lai and colleagues investigated whether there was a connection between a decrease in M cells and the extent of SFB colonization of the Peyer's patches. The authors demonstrated that M-cell depletion mediated by nociceptors, or triggered through an antibody-mediated experimental approach, led to an increase in this colonization, suggesting that the number of M cells can modulate SFB colonization in the gut (although the exact mechanisms responsible were not fully determined). This outcome was beneficial because it limited *Salmonella* infection, presumably because the higher presence of SFB and the depletion of M cells together resulted in a reduction of invasion sites available for *Salmonella*. Finally, Lai *et al*. report that when TRPV1-expressing nociceptors encountered *Salmonella*, the neurons released a neuropeptide called CGRP. This small molecule enables communication between cells. CGRP was directly able to regulate M-cell abundance and function, as well as to regulate SFB levels in the gut.

The authors have uncovered a previously unrecognized role for nociceptors in host defence against *Salmonella* infection. These remarkable findings reveal a complex loop of interactions between epithelial cells, neurons and microbes in the mammalian gut, adding another layer of complexity to our understanding of gut immunity. Whether nociceptor-mediated responses help to defend against a variety of other microbial pathogens remains to be determined. Indeed, nociceptors have been reported to protect mice during infection by the bacterial pathogen *Citrobacter rodentium*[14].

A key area for future investigation will be to determine whether Lai and colleagues' findings have relevance for human health. For example, one area that would be worth studying is whether long-term use of pain-blocking opioid drugs, such as morphine, might affect nociceptor-mediated antibacterial defence. This is of interest because nociceptors are the main target of opioids, and administering morphine to mice changes the gut's microbial composition[15,16]. Moreover, morphine use promotes the spread of certain types of microbe (Gram-negative bacteria) from the gut to elsewhere in the body, a process that can lead to sepsis, a potentially life-threatening immune response to infection[15,16]. Future research that explores interactions between neurons and immune cells during infection could uncover further exciting findings that will profoundly influence our understanding of host defence.

"These remarkable findings reveal a complex loop of interactions between epithelial cells, neurons and microbes."

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Cancer genetics

Not all driver mutations are equal

Victoria L. Bae-Jump & Douglas A. Levine

A study of cancer-associated mutations in normal endometrial glands of the uterus has now been performed using whole-genome sequencing. The analysis sheds light on the early changes that lead to invasive disease. **See p.640**

Understanding how normal tissues give rise to cancer is crucial for improving prevention and early detection of this deadly disease. Over the past two decades, the genomic profiles of most types of invasive cancer have been catalogued; however, similar profiling of normal tissues presents a unique set of challenges. Cancer tissues are often abundantly available from biopsies or surgery, but samples from normal tissues tend to be much smaller, and specimen-collection practices are less well established, making it hard to gather high-quality material. Moore *et al*.[1] overcome these challenges on page 640, and successfully catalogue cancer-driving mutations in normal endometrial glands.

Endometrial glands are abundant in the lining of the uterus, where they secrete hormones and other substances that are essential for normal menstruation and embryonic development. Endometrial cancer is the sixth most common cancer in women worldwide, with more than 382,000 cases annually[2]. The mortality rate has increased over the past decade[3], heightening the need for prevention and early detection of this disease.

Moore *et al.* obtained 257 normal endometrial glands from 28 women of various ages. In each case, the authors meticulously isolated the glands using a technique called laser-capture microdissection to separate the epithelial tissue, which lines the gland, from the surrounding stromal cells that make up the gland's connective tissue. They then performed whole-genome sequencing of the epithelial samples and various other normal tissues from the same women, using a protocol they had developed that is tailored to the analysis of small amounts of DNA.

The group analysed these sequences to identify mutations that are unique to the normal endometrial glands, as well as endometrium-specific changes in the number of copies of any genetic region (caused by duplication or deletion of DNA). They found that, in almost 90% of individuals, the normal endometrial tissue contained driver mutations — which give cells a selective advantage over non-mutated counterparts, and so are thought to promote cancer development. Nearly 60% of the endometrial glands in these women contained one or more drivers.

The authors found 12 genes that contained driver mutations with statistically increased prevalence in normal endometrial tissue compared with that in other tissues. These genes are all known to be frequently mutated in cancer, and, collectively, these mutations have the potential to affect many cellular processes. However, isolated mutations in the individual genes, as was typically the case in Moore and colleagues' samples, are probably insufficient to make a tissue become cancerous[4].

A remarkable finding is that each endometrial gland seems to be clonal — that is, all the cells in the gland are derived from a single epithelial progenitor cell. It might be expected that each gland could develop multiple independent mutations, but the authors' discovery of clonality indicates that there is instead a uniformity to the mutational process.

As would be expected, the number of mutations increased with age, at the rate of about 29 nucleotide substitutions per gland per year during adult life. Moore *et al.* reconstructed the phylogeny (the evolutionary development and diversification) of individual glands to document the initial presentation and spread of driver mutations through the tissue over time. They report that many glands that were located in close physical proximity in the uterine wall displayed distant phylogeny. This suggests that the cellular populations in each gland remain genetically isolated, providing many separate opportunities for cancer to develop. The researchers also provide evidence that driver mutations can arise at any time, occurring in some women in their first decade of life and in others at various stages of adulthood. This insight is important because the typical timeline between developing driver mutations and cancer is not yet well defined.

The group's rigorous methods for sample isolation and sequencing, coupled with their well-developed bioinformatics algorithms, mean that the results of this study should be highly reliable and reproducible. But one caveat is that the authors isolated endometrial glands from a select population of women: most samples were obtained from people undergoing evaluation for infertility, from organ donors, or from women who had died of non-gynaecological causes. Both infertility and nulliparity (having never given birth) are known independent risk factors for endometrial cancer[5]. And samples collected from women who had died of non-gynaecological causes might be more likely than the average endometrial gland to contain low-risk driver mutations that have less potential to trigger cancer, given that these women died without having developed endometrial cancer.

Future studies would benefit from a more-representative cross-sectional population. The inclusion of women who have conditions that are well-known precursors to cancer, such as atypical endometrial hyperplasia (in which the lining of the uterus becomes abnormally thick) could help in this regard. Researchers might then be able to define a robust landscape of changes that occur during the progression from normal to precancerous tissue to invasive disease. This approach might also help to define the pathogenicity of, and possible necessity for, individual driver mutations that lead to the development of cancer.

"The authors' findings should be useful for ongoing research to detect endometrial cancer at early stages."

Another caveat is the discrepancy between driver mutations identified by Moore *et al.* and those from other cancer-genome projects, including The Cancer Genome Atlas[6]. Although the most frequently mutated genes identified in the current study have been previously reported in endometrial cancers, several of the most commonly mutated genes in this cancer are notably not mutated in Moore and colleagues' samples. The group found mutations in these well-known drivers in less than 2% of the normal endometrial glands that they studied — a surprisingly low frequency, because one would expect that the drivers present in all cancer cells would be the first to arise in normal tissue. This discrepancy probably hints at unknown aspects of the multistep process of tumour initiation, in which certain mutations must arise before others. Determining when and how gatekeeper mutations occur and permit the next step in tumour development will require further analyses of benign, premalignant and invasive tissues.

Knowing that the compilation of driver mutations in normal endometrial glands is different from those found in established endometrial cancers might change the approach for further research into the prevention and early detection of this disease. Determining the role of these mutations in concert with other known risk factors, such as nulliparity, obesity, race and genetic predisposition, will help to better identify women who are at risk of endometrial cancer. Even before we obtain this information, Moore and colleagues' findings should be useful for ongoing research to detect endometrial cancer at early stages, which includes analyses of cell-free DNA circulating in blood, tampon-based collection of vaginal secretions and liquid-based examination of cervical tissues[7–9]. More broadly, a better overall understanding of the normal mutational spectra in tissues will refine our knowledge of the consequences of specific cancer drivers for many solid tumours.

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