

Modelling western dietary habits in the mouse: easier said than done

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The recent debate on the relative importance of environmental *vs.* intrinsic factors in the onset of malignancy has raised major concerns that the general public might conclude that cancer prevention programmes are not worthwhile. In their original report (1), Tomasetti and Vogelstein concluded that a majority of cancers can be explained by the high number of stem cell divisions in tissues with a high cell turn-over, due to generation of random mutations and their accumulation each time DNA replicates. In a subsequent report (2), Wu and colleagues employed a different mathematical model and extended the data set to reach the opposing conclusion that spontaneous mutations occurring during stem cell division rarely reach the level necessary to underlie cancer development. In the majority of cases, exposure to environmental risk factors represents a fundamental requirement for the onset of malignant disease. Of note, in the mathematical approach of the former study (1), the intrinsic rate of stem cell division and the environmental risk factors were regarded as entirely independent variables whereas it is plausible to think that extrinsic factors do affect stem cell homeostasis.

From this perspective, the study by Beyaz *et al.* (3) on the effects of a high-fat diet (HFD) on *Lgr5*⁺ intestinal stem cells is of high relevance. Colon cancer, *i.e.*, one of the most common malignancies in western populations, has been indisputably shown by epidemiological and animal studies to have a strong environmental component, with long-term western style dietary habits among the major etiological

risk factors. By employing mouse models (wild type and *Apc*-mutant) fed with an obesity-inducing HFD, Beyaz and colleagues concluded that, through activation of PPAR- δ among other signalling pathways, the HFD not only alters the function of both intestinal stem (*Lgr5*^{hi}) and progenitor (*Lgr5*^{low}) cells, but also enhances their capacity to trigger tumour formation (3).

The relevance of mouse studies for our understanding of the cellular and molecular mechanisms through which specific nutrients underlie colon cancer onset is largely dependent on our capacity to model western-style dietary habits *in vivo* with appropriate controls. From this perspective the diets employed in the Beyaz study raise a number of serious concerns.

First, the control diet is a conventional “chow” (LabDiet, RMH3400) whereas the HFD is a purified product (Research Diets, D12492) produced by a different manufacturer and entirely different in nutrient levels when compared with the control diet. Of note, chow diets are typically used for ‘maintenance’ purposes because they provide complete and adequate nutrition at a relatively inexpensive price. However, they encompass plant-derived ingredients and are inappropriate for nutrition studies because of the high-dosage and variable levels of plant phytoestrogens. In chow-fed mice, serum phytoestrogens reach levels several orders of magnitude higher than endogenous estrogen, but are undetectable in animals fed with a purified diet (4,5). The substantial increase

in phytoestrogens is likely to alter multiple signaling and metabolic pathways in chow-fed control mice when compared with HFD-fed mice. Therefore, it becomes impossible to attribute the dietary effects to any specific component of the experimental diet.

Second, besides the critical dietary issues stated above, both chow and purified diets provide levels of vitamin D3 (2.5 and 1.0 IU/g for control and HFD, respectively), establishing 25(OH)D serum levels well above those reported for the US population (http://www.cdc.gov/nchs/nhanes/nhanes_products.htm). The elevated vitamin D3 level in both diets not only fails to reflect the dietary habits of at risk individuals in the population at large, but is also likely to have profound implications on stem cell biology. Previously, a signature of genes specifically expressed in Lgr5^{hi} stem cells was established that includes the vitamin D receptor (*Vdr*) gene. Of note, *Vdr* is down-regulated when Lgr5^{low} cells exit the stem cell niche (6), thus suggesting that Lgr5^{hi} cells may depend on vitamin D signalling for their stem cell function, as also previously observed in other stem cell niches (7,8). This was confirmed by feeding mice with a purified western style diet (NWD1) characterised by low vitamin D levels mirroring individuals at high risk for colon cancer (9). Accordingly, *in vivo* inactivation of the *Vdr* gene in Lgr5^{hi} cells compromises their ability to lineage trace whereas lower dietary vitamin D3 affects both their stem cell function and their capacity to form tumours upon targeted mutation of the *Apc* gene (9). In view of these data and of previous observations substantiating the “top-down” model of intestinal tumour formation, other stem cell compartments, as well as differentiated cell types are likely capable, upon stress factors of various nature, of re-entering the cell cycle and of acquiring stem cell characteristics as part of the tissue adaptive response to this particular environmental factor, as well as perhaps others (10,11).

The notion that Lgr5^{hi} cells do not efficiently function as intestinal stem cells at vitamin D3 levels characteristic of the vast majority of the human population clearly and inevitably affect not only the conclusions reached by the Beyaz *et al.* study relative to the changes in these cells as the cause of high fat/obesity increased colon cancer incidence in the human population, but those of many other mouse studies of intestinal stem cell biology based on standard chow diets. Modelling human dietary habits in the mouse requires the use of properly controlled defined or purified diets that well translate to the human, as frequently discussed in the scientific literature (12-14). Mouse models may remain relatively poor surrogates for many human diseases

and drug discovery until cell and molecular biologists understand the profound impact of well-controlled diets in establishing cell, tissue and organ physiology.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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