PLENARY LECTURE

Normal and leukemic stem cells

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Studies on hematopoietic stem cells have provided several critical insights in the biology of stem cells in general; as mature blood cells are generally short lived, stem cells are in fact required to guarantee, throughout the life of an organism, the replenishment of differentiated blood cells by the generation of multi-lineage progenitors and precursors committed to individual hematopoietic lineages. Similarly, acute myeloid leukemia has been considered as a model system to study cancer stem cells. This presentation illustrates some recent results obtained by our group with regard to both normal and leukemic stem cells.

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Tumors have been regarded as morphologically heterogeneous cellular structures since the early nineteenth century; however, scientists began to investigate the functional meaning of this heterogeneity only several decades later. *In vitro* and *in vivo* studies showed that, within a given leukemia sample, very few cells possess clonogenic activity *in vitro* and the ability to transplant the disease in secondary hosts. These findings, although not directly proving the existence of leukemic stem cells (LSCs), indicated that only a restricted population of cells might have a functional role in the growth and maintenance of leukemia. Stronger evidence of the existence of LSCs arrived more recently, with the development of advanced flow cytometry methods and new xeno-transplantation models. Although rare, in the order of 1×10^5 – 10^6 , LSCs also are a functionally heterogeneous cell population, consisting of distinct classes of cells with different self-renewal potential and, similar to normal hematopoietic stem cells (HSCs), distinguishable into 'long-' and 'short-repopulating' cells on the basis of their capacity to perpetuate the leukemic clone during serial transplantation assays.

Recently, we showed that the cell cycle inhibitor p21 is crucial to the self-renewal maintenance of mouse leukemic and preleukemic stem cells (that is, HSCs expressing leukemic fusion proteins).¹ We found that the expression of leukemia-associated oncogenes in HSCs induces DNA damage and upregulation of p21, activating a cellular response that leads to reversible cell cycle arrest (quiescence) and DNA damage repair. Moreover, we discovered that, in the absence of p21, preleukemic stem cells are more sensitive to proliferative stress than normal stem cells, eventually losing their capacity for self-renewal and becoming depleted. In fact, although the leukemia-associated oncogene PML-RARA induces leukemia both in the presence or in the absence of p21, p21-null leukemias are not transplantable, suggesting a defect in LSC self-renewal. This defective behavior correlates with active recruitment of LSCs into the cell cycle and a drastic reduction in their number. In addition, AML1-ETO, another leukemia-associated oncogene, cannot induce leukemia in p21-null animals. Thus, loss of p21 affects the self-renewal of LSCs.

The capacity of a cell to repair DNA is essential to the integrity of its genome, which is subjected to continuous environmental assaults and to errors in DNA replication during cell division; any

resulting damage, when repaired, might lead to accumulation of DNA mutations and, ultimately, to diseases such as cancer. Therefore, cells have evolved a number of mechanisms to detect and repair the various types of DNA damage. In addition, adult proliferating cells deal with excessive accumulation of genomic damage with the elimination of the damaged cell through apoptosis or senescence, processes mediated by the tumor suppressor p53 (which is, not surprisingly, almost always inactivated in spontaneous tumors) and its transcriptional target p21.

Stem cells, however, appear resistant to many genetic insults, perhaps through the selection of specific DNA damage repair mechanisms that allow their survival and the regeneration of injured tissues. How do SCs carry out their response to DNA damage and concomitantly suppress tumorigenesis? Being 'quiescent' or 'infrequently dividing' is generally considered a distinctive property of normal SCs. HSCs are resistant to anti-proliferative chemotherapeutic agents such as fluorouracil (5-FU), and this is probably due to their being, mostly, non-proliferating cells. Indeed, a quiescent state seems essential for their activity, and HSC hyper-proliferation results in functional exhaustion. Mechanistically, it is assumed that accumulation of genomic damage by HSCs during DNA replication is the cause of this functional exhaustion. Ultimately, the genomic damage will lead to DNA mutations, causing progressive loss of typical SC functions, such as their replication capacity. It is unclear, however, whether quiescence is the mechanism by which HSCs protect their self-renewal property and repair DNA damage, preventing transformation.

We have thus studied the role of p53 and p21 in DNA damage repair in highly purified murine HSCs after X-ray treatment. Our data suggest that different response mechanisms to DNA damage are present in long-term reconstituting HSCs (LT-HSCs) with respect to progenitor cells (multipotent progenitors and common myeloid progenitors). These mechanisms appear to be dependent on the activation of p21 and are also operational in the absence of p53 (as, for instance, in p53-null HSCs). Specifically, in LT-HSCs, upregulation of p21 inhibits activation of p53 (an event that is usually typical in the first stages of the response of more mature cells to excessive genomic damage) and thus prevents apoptosis or senescence. As DNA is a molecule that has an active and crucial

role in cell division, DNA repair is closely linked to cell cycle regulation. p21 appears to be involved in the transient cell cycle arrest associated with moderate/low levels of DNA damage in different cell types; thus, in order to investigate how p21 can prevent accumulation of genomic damage, we analyzed the cell cycle properties of LT-HSCs after X-rays. We found that X-ray exposure induces recruitment of LT-HSCs into the cell cycle and expands their absolute number: importantly, both effects depend on p21.

Collectively, our data suggest that the progressive loss of self-renewing potential, from LT-HSCs to progenitors, correlates with different DNA damage repair mechanisms. Crucial to the response of LT-HSCs is the activation of p21, which allows repair and results in the expansion of a pool of functional stem cells to limit the exhaustion of HSCs, and it is perhaps due to the ability of p21 to inhibit p53; we know in fact that p53 prevents symmetric divisions in mammary SCs.² However, p53 does not appear to be a key player in the DNA damage response of HSCs. We are now trying to determine whether pathways similar to those described in normal HSCs are also active in normal SCs from other tissues.

Finally, our results have implication for cancer therapy. Increasing evidence suggests that cancer stem cells (CSCs) are largely chemo- and radioresistant, thus surviving *in vivo* treatments and initiating tumor regrowth. Is p21 also implicated in

CSC resistance to therapy and relapse? Our recent findings on oncogene-induced DNA damage and upregulation of p21 in HSCs¹ seem to suggest so, and, given that the efficiency of DNA repair appears linked to the self-renewal ability of LSCs, indicate novel therapeutic approaches.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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REFERENCES

- 1 Viale A, De Franco F, Orleth A, Cambiaghi V, Giuliani V, Bossi D *et al.* Cell-cycle restriction limits DNA damage and maintains self-renewal of leukaemia stem cells. *Nature* 2009; **457**: 51–56.
- 2 Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, Giulini B *et al.* The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell* 2009; **138**: 1083–1095.