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On clonogenic tumour cells and metastasis-forming cells

Maurice Tubiana and Serge Koscielny

We read with interest the paper by Michael Baumann, Mechthild Krause and Richard Hill on cancer stem cells (Exploring the role of cancer stem cells in radioresistance. *Nature Rev. Cancer* **8**, 545–554 (2008))[1]. We would like to make two remarks.

First, the authors list the terms used for designating cancer stem cells. However, they do not include those from the 1950s to the 1970s — colony-forming units and clonogenic cells — whereas a large number of papers used these terms. For example, we showed that the proportion of clonogenic cells in S phase can be determined by *in vitro* incubation with hydroxyurea[2–4]. The proliferation rate of clonogenic cells in a mammary tumour was measured following administration of hydroxyurea (5 mg per minute) or ionizing radiation (0.3 Gy). The changes in the labelling index and the mitotic index of the tumour cell population were different from those of the proportion of clonogenic cells in S phase. These differences were derived from differences between the cell cycle times of the clonogenic and the non-clonogenic tumour cell[3,4]. In addition, a significant recruitment of quiescent clonogenic cells into the proliferative compartment about 5 hours after administration of hydroxyurea was observed[4]. After irradiation, blocks at G1/S and S/G2 phases of the cell cycle induced a semi-synchronization of clonogenic tumour cells that lasted approximately two cell cycles. These studies remain valid and are probably relevant for cancer stem cells.

Second, we studied the proportion of stem cells in the population of tumour cells in human breast cancer during tumour growth[5–7]. We assumed that the probability of distant metastatic dissemination during tumour growth is correlated to the number of metastasis-forming cells (probably stem cells). In tumours 10 mm in diameter the maximum number of cells is 0.5×10^9 (REF. 7). Metastases were present or developed after the removal of the tumour in 11% of the patients[5,6]. This corresponds to a probability of distant metastatic dissemination of 20% per 10^9 cells during this period.

For tumours from 10 to 20 mm in diameter, the maximal number of cells is 4.2×10^9 and the proportion of patients with metastases increased by 16%. During this growth from 0.5×10^9 cells to 4.2×10^9 cells, the probability of distant dissemination is 3.9% per 10^9 cells. For tumours from 20 to 30 mm in diameter, that is from 4.2×10^9 to 14.1×10^9 cells, the number of patients with metastases increased by 13% and the probability per 10^9 cells is 0.9%. During the growth from 30 mm to 40 mm (14.1×10^9 and 33.5×10^9 cells) these values are 10% and 0.3%, respectively. The proportion of metastasis-forming cells further decreases to 0.3% and to 0.1% for tumours 40 and 50mm in diameter. Hence the proportion of metastasis-forming cells is much higher in small tumours and their number is nearly constant thereafter. For tumours of less than 30 mm in diameter the proportion of metastasis-forming cells is about 2-fold higher in grade 2 tumours than in grade 1, and slightly greater in grade 3 tumours.

Thus, the proportion of stem cells varies widely with various factors, which should be taken into account when discussing therapeutic implications.

Maurice Tubiana is at the Gustave Roussy Institute, Villejuif, France and Serge Koscielny is at the Department of Epidemiology, Gustave Roussy Institute, Villejuif, France.
Correspondence to M.T.
e-mail: maurice.tubiana@univ-paris5.fr

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