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Metabolic Aspects of Cell Cycle Regulation in Normal and Cancer Cells*

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ABSTRACT

Several studies are reviewed dealing with the mechanisms which regulate the cell cycle progression in normal and cancer cells. Using Yoshida AH 130 ascites tumor cells, it has been found that the G_1-S transition of these cells is impaired by specific inhibitors of the electron flow through the respiratory chain (antimycin A), although respiratory ATP can be replaced by glycolytic ATP. The above transition can be also inhibited by the addition of physiologic substrates, mainly pyruvate, by a mechanism which appears linked to a modification of the cellular redox state and can be totally reversed by adding adenine to the culture medium. Adenine equally removes the block produced by antimycin A, pointing out a respiration-linked step of purine metabolism restricting the cell recruitment into S. A substantial protection of this step against the inhibitory effects of pyruvate and antimycin A has been obtained by the addition of folate and tetrahydrofolate, suggesting that the respiration-linked limiting step of tumor cell cycling involves folate metabolism and its connection to purine synthesis. The biologic relevance of these findings is stressed by the fact that pyruvate addition also inhibits the proliferation of concanavalin A-stimulated lymphocytes as well as of bone marrow hemopoietic cells in the presence of colony-stimulating factors. On the other hand, pyruvate only slightly affects the growth kinetics of malignant lymphoblasts and of Friend erythroleukemia cells either in the absence or in the presence of the differentiation inducer dimethylsulfoxide.

INTRODUCTION

The membrane-mediated modulation of the mitotic cycle tends to disappear in cancer cells, so that this cycle can be triggered independently of the "growth factors" (2), which initiate on the cell surface the complex series of events culminating in DNA synthesis and mitosis (the *pleiotypic response*) (17). The loss of this modulation does not mean, however, that cancer cells have no possibility of controlling the mitotic cycle. On the contrary, tumor cells manifest a remarkable attitude to arrest in G_1, surviving for prolonged periods (even for years) in conditions of growth restraint and thus frustrating radio- and chemotherapy treatments based on cell cycling. The establishment and maintenance of this "dormant state" of cancer (26) is in many cases due to oxygen and nutrient shortage in the environment (22), and termination of this state promptly occurs when tumor silent foci autoinduce neovascularization (11). It is established now that oxygen deprivation is decisive for the cells to arrest their growth (4, 5, 10, 13–16, 22, 23), entering in several cases the dormant state, while anaerobic glucose supply can only afford cell sur-

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vival. To explain this fact, contradicting the old tenet of Warburg that cancer cells can grow at the expense of glycolysis only (24), the assumption is usually made that some energetic barriers exist along G_1, which cannot be overcome without the contribution of the respiratory ATP (23, 25). According to our studies, this assumption proved untenable, and evidence has been obtained that the essential role of respiration in allowing the G_1-S transition of tumor cells relies on a much more sophisticated connection between the mechanism of cell cycle regulation and the cellular redox state.

EXPERIMENTAL PROCEDURES AND COMMENTS

Our studies started with an extremely anaplastic tumor cell population (Yoshida AH 130 ascites hepatoma), which at the plateau of tumor growth *in vivo* arrests in G_1, due to oxygen shortage (8), maintaining a high rate of protein turnover (19). Removal of G_1 block and cell recruitment into the S phase can be produced by incubating the cells in air in a culture medium made by diluting the autologous ascites plasma with a buffered saline containing 15 mM glucose (20). This recruitment can be easily monitored by measuring the rate of [^{14}C]thymidine incorporation into DNA of the cell population, an index proved directly proportional to the number of cells in S at the corresponding times, as measured by autoradiographic techniques (20). The substitution of air with nitrogen atmosphere completely abolished the G_1-S transition without affecting the rate of lysine incorporation of ascites cells, indicating that a respiration-linked limiting step regulates the above transition without influencing other important energy-dependent processes.

In order to enlighten this regulatory step we tried to distinguish the effects of blocking the electron flow to oxygen from those of uncoupling this flow from ATP production, by using selective inhibitors of these processes such as antimycin A and 2,4-dinitrophenol (DNP), respectively (21). Results showed that antimycin A inhibits the cell recruitment into S, while DNP, at concentrations blocking the production of mitochondrial ATP and hence enhancing electron flow to oxygen, has no inhibitory effects on this recruitment (21). Neither antimycin A nor DNP substantially affected the rate of [^{3}H]-lysine incorporation into proteins, confirming that, provided glucose is available, protein synthesis is not depressed when respiratory ATP supply is abolished. Direct measurement of ATP content showed that neither antimycin A nor DNP induce any change in the overall ATP content of ascites cells, due to a compensatory increase of the rate of glycolysis (21). At this point it was possible to conclude that the tumor cell transition from the noncycling to the cycling state strictly depends on respiration as far as some limiting step of this transition requires the oxidation of reducing equivalents through the respiratory chain, while ATP requirement for cell recruitment into S can be totally afforded by glycolysis only.

As a first attempt to characterize this step, it was decided to study the effects of the addition of various oxidizable substrates to the system. In this context it was known that, by adding an excess of these substrates to tumor cells, their oxidation is forced at the expense of the endogenous substrates, which already drive respiration at its maximum rate (1, 9). Under these conditions, interfering with the oxidation of endogenous metabolites by adding an excess of different oxidizable substrates seemed to offer a way to gather information about the respiration-linked limiting step in our system. Results showed that cell recruitment into S is unaffected by the addition of 10 mM α-ketoglutarate, succinate, fumarate, aspartate, malate, glutamate, and lactate, while it is inhibited 80% and 50% by 10 mM pyruvate and oxalacetate, respectively (18). A slight but significant inhibition is also exerted by citrate and isocitrate. Thus, the G_1-S transition of tumor cells can be abolished by either blocking the respiratory chain or forcing the oxidation of some physiologic substrates, mainly pyruvate. The analysis of the mechanism of pyruvate inhibition showed that the latter is not attributable to specific interferences of this substrate with the citric acid cycle or glycolysis, while it closely mimics the effects of antimycin A on both cell recruitment into S and the rate of lysine incorporation (18). Furthermore, through a complex series of experiments, it was possible to demonstrate that pyruvate inhibition of the G_1-S transition can be totally removed by adding preformed adenine to the system. Removal was also obtained by adding guanine, but not pyrimidine bases, pointing out the peculiarity of purines in antagonizing the pyruvate inhibition. Finally, adenine removes the inhibition of the G_1-S transition

produced by antimycin A, indicating that a similar mechanism underlies the restriction of this transition produced by antimycin A and by an excess of oxidizable substrates (18).

Altogether these results lead to the metabolic schema illustrated in Figure 1. The G_1-S transition of tumor cells requires an oxidative step along the synthesis of the purine ring, driving reducing equivalents onto the mitochondrial respiratory chain. This step is therefore inhibited by substances blocking the electron flow through this chain (antimycin A), while, provided glucose is available, it is not affected by uncoupling agents (DNP), which either do not modify or enhance the rate of this flow. The inhibition of the cell recruitment into S consequent to the block of respiration can be by-passed by adding preformed purines, principally adenine, which in fact relieves the antimycin A block. However, a similar adenine-reversible block can be obtained any time the respiratory chain is monopolized or saturated by an excess of oxidizable substrates not related to the limiting step, the oxidation of which can be brought about at sufficiently high levels. Depending on the activities of the dehydrogenases involved in this oxidation, the nature of these substrates may vary from one type of tumor cell to another. However, the saturation of the respiratory chain is especially liable to occur in cells having a modest oxidative capacity, such as highly anaplastic tumor cells (1) or in hypoxic environments.

The nature of the oxidative step limiting the purine synthesis and hence the G_1-S transition remains at the moment fairly speculative. However, since a complex series of NAD or NADP-dependent interconversions regulate the production of an essential factor for purine synthesis, namely N^5,N^{10}-methenyltetrahydrofolate (12), it is reasonable to hypothesize that these interconversions represent the processes connecting purine synthesis to the cellular redox state governed by respiration. This hypothesis is supported by

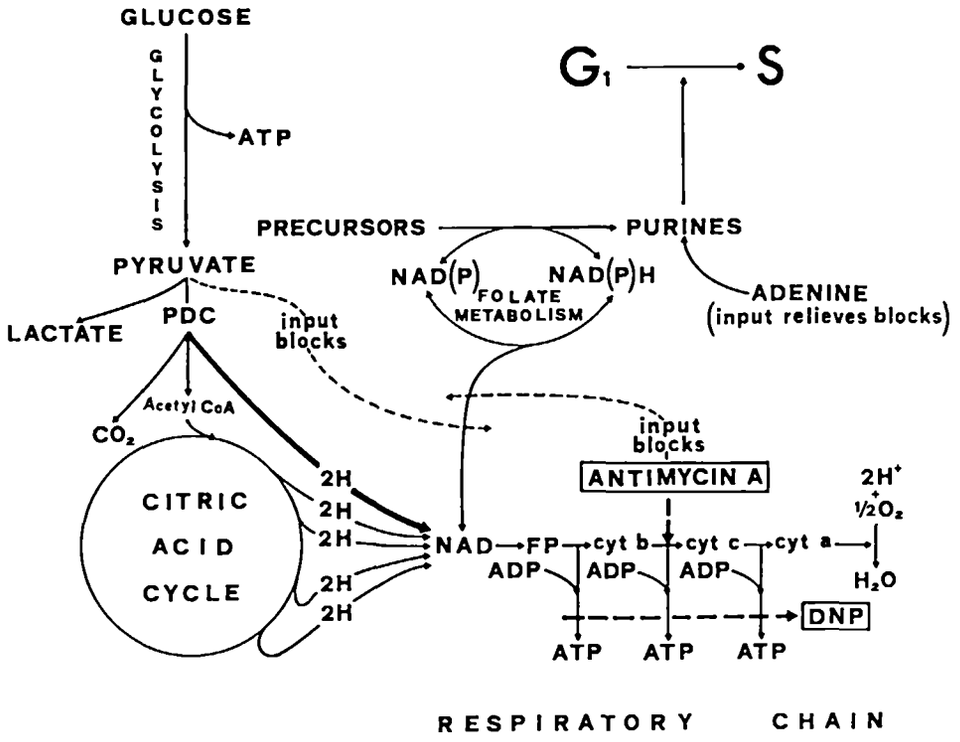

FIG. 1—Diagram indicating the postulated redox mechanism controlling the G_1-S transition of tumor cells. This transition is represented as a process restricted by an oxidative step connected with purine synthesis (possibly the NAD(P)-dependent interconversion of folate metabolism), driving reducing equivalents onto the respiratory chain. This limiting step is inhibited by either blocking the electron flow to oxygen (antimycin A) or by saturating this chain by reducing equivalents produced in the oxidation of unrelated substrates (pyruvate). Both these blocks are relieved by the addition of preformed purines (adenine). Provided glucose is available, the abolition of respiratory ATP without impairing the electron flow (DNP) does not inhibit the oxidative step limiting purine synthesis and has no effects on the G_1-S transition. PDC, pyruvate dehydrogenase complex; FP, flavoprotein; cyt, cytochrome.

the fact that addition of suitable concentrations of folate and tetrahydrofolate to ascites cells in our system removes substantially the inhibition of the G_1-S transition brought about by pyruvate and antimycin A (18). On the whole, a connection between folate metabolism and cell progression through the mitotic cycle emerges as the extreme link maintaining the respiration-dependence of cell growth in mammalian cells even at the highest degrees of anaplasia. However, further studies showed that this regulatory mechanism is not peculiar to anaplastic cells, but it is shared by normal cell populations having the following characteristics: i) the capability of long survival in a quiescent state (G_0); ii) a strong clonogenic potential; iii) a high rate of aerobic glycolysis. In fact a marked inhibition of the G_1-S transition is brought about by pyruvate in at least two types of these populations, namely mouse T lymphocytes proliferating in response to concanavalin A (6) and bone marrow stem cells stimulated *in vitro* by colony-stimulating factors (7). On the other hand, two types of murine leukemia cells were found scarcely sensitive to the inhibitory effects of pyruvate on cell cycle progression. In fact, up to 20 mM, this substrate does not significantly affect the kinetics of growth *in vitro* of a radiation-induced CBA mouse leukemia or the growth of Friend leukemia cells either in the absence or in the presence of the differentiation inducer dimethyl sulfoxide (3). Since, however, both these types of leukemia cells cannot grow when respiration is impaired, it remains uncertain whether in these cells pyruvate cannot be oxidized at a rate saturating the respiratory chain or the connection between growth and respiration is mediated by an alternative mechanism to that illustrated above.

Concluding Remarks

The respiration-dependent restriction of the cell cycle progression and its link to the folate metabolism have some practical and theoretical implications which deserve further remarks.

First of all, whatever the final link between purine metabolism and the respiration-dependent limiting step of cell recruitment into S, this link may represent an important mechanism by which tumor cells arrest in G_1 in hypoxic areas (22), devoting their metabolism to survival rather than to replication and thus entering the dormant state. Understanding of this mechanism may aid to solve the problem of tumor survival in silent foci.

Another important point raised by our studies concerns the cytostatic effects exploited by physiologic substrates, mainly pyruvate, through their interferences with the redox steps of purine metabolism. In fact, the possibility remains to be explored that the supply of these substrates improves the efficacy and the specificity of cancer treatments with purine analogs or antifolic agents, at least when dealing with anaplastic tumors characterized by high rates of glycolysis and scarce oxidative capacity. As far as the growth of these tumors is limited by the respiration-linked step of purine metabolism, the effects of saturating the respiratory chain by some highly oxidizable substrates should selectively potentiate the block of purine synthesis operated by the chemotherapeutic agents.

Finally, the ability of pyruvate to block the G_1-S transition of tumor cells may throw some light on the controversial role of aerobic glycolysis of these cells. In fact, in view of the cytostatic properties of this substrate, the essential feature of aerobic glycolysis, i.e., pyruvate conversion to lactate even in the presence of oxygen (24), appears as a propitious feature for growth, as lactate may be accumulated in the medium without affecting the G_1-S transition (18). Any time the oxidative capacity of the cells undergoes a severe limitation, the above conversion may be decisive to allow a high glucose breakdown, without creating the impairment of cell recruitment into S which would result from pyruvate oxidation through the citric acid cycle. The latter may represent an important mechanism to permit cell replication in hypoxic areas, maintaining the respiratory chain available for the oxidative step connected with purine metabolism or other essential pathways.

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