On the Clinical Significance of the S-Phase Fractions of Tumors*

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ABSTRACT

The S-phase fraction of a tumor cell population is often viewed as a general indicator of the clinical aggressiveness of that tumor. Actually, the S-phase fraction of a cancer should be interpreted as no more than an indicator of the mean duration of its mitotic cycle. The S-phase fraction of a solid tumor is difficult to measure accurately, but in principle it is a powerful predictor of the duration of the recurrence-free interval. However, since the duration of the mitotic cycle does not correlate with malignancy, the S-phase fraction cannot be used to diagnose malignancy. It is of little value in making therapeutic decisions because the duration of the mitotic cycle is not a predictor of invasion and metastasis.

Introduction

The S-phase fraction of a human cancer was first measured 35 years ago, and since then there has been a growing interest in its relation to the biological behavior of tumors. As cellular deoxyribonucleic acid (DNA) histograms have become easily available through the clinical use of flow cytometry, the S-phase fraction has been routinely reported as part of the prognostic profile of tumors. As such, it has been interpreted as a general indicator of the aggressiveness of tumors and, especially in the case of breast carcinomas, it would seem to provide a basis for decisions about chemotherapy. The following is a discussion of just what the S-phase fraction may or may not indicate about the biological behavior of solid tumors.

The Cell Cycle

In its mitotic cycle the cell passes through four recognizable phases based upon its DNA content.
1. The time spent in the diploid state is called the first gap-phase, the G1 phase. During this phase the DNA complement remains constant, but there is cytoplasmic growth so that each doubling of the population does not result in a progressive decrease in average cell size.
2. The time during which DNA synthesis takes place is called the S-phase. At any particular moment during this phase the DNA complement will be somewhere between diploid and tetraploid.
3. The time spent in the tetraploid state, the gap between DNA synthesis and mitosis, is the second gap, the G2-phase.
4. The time spent in mitosis is called the M-phase. As the cell divides its DNA complement falls from tetraploid to diploid.

In principle the cell population should double during the period of one mitotic cycle, i.e., the average time required for a cell to proceed from one mitosis to the next. In reality, the doubling time of a cell population, or the doubling time of the mass of a tumor, is longer than the period of the mitotic cycle, leading to the conclusion that not all cells completing one mitosis enter the next mitosis on schedule. This can be due to one of three things. A cell may die before reaching the next mitosis (an important factor in tumors with extensive necrosis), a cell may take a temporary time out before re-entering the mitotic cycle, or a cell may mature, leaving the proliferating pool permanently. Viable cells that are not in the mitotic cycle are said to be in G₀-phase.

Estimating Tumor Growth Rate from the S-Phase Fraction

In the case of a steady state population with a negligible G₀ subgroup, the fraction of a cell population in a given phase at any given moment is proportional to the duration of that phase. This is only approximately true of a growing cell population. For example, if the duration of identifiable mitosis is one hour and one per cent of the population is found to be in mitosis at a given moment, then one hour is about one percent of the duration of the entire mitotic cycle, and the duration of the mitotic cycle is about 100 hours.

Accordingly, the S-phase fraction, Fₛ, can be approximated by

\[ F_s = T_s/T_c \]

and

\[ T_c = T_s/F_s \]

where \( T_s \) is the duration of the S-phase, and \( T_c \) is the duration of the entire mitotic cycle.

For an exponentially growing population, the ratio of the number of cells at time \( t \) to the initial number of cells is

\[ N_t/N_0 = \exp(R t) \]

where \( R \) is the growth rate constant.

Thus, after one mitotic cycle,

\[ 2 = \exp(R T_c) \]

and

\[ R = \ln 2/T_c \]

Combining (1), (2) and (3), the number of cells at time \( t \) is

\[ N_t = N_0 \exp[(t F_s \ln 2)/T_s] \]

\( T_s \), the duration of the S-phase, can be measured in a tedious way by measuring the time taken for pulse labeled S-phase cells to pass by a given point in the cell cycle. By measuring, at various times after pulse labeling of S-phase cells, the fraction of labeled mitotic figures, one can measure the time spread of S-phase cells and thus the approximate duration of the S-phase (figure 1). This has been done in several mammalian tissues and solid tumors and usually indicates an S-phase duration of about 5 to 8 hours.

S-Phase Fractions of Carcinomas of the Breast

Measurements of S-phase fractions of solid tumors are subject to large sources of error. The S-phase fraction is commonly reported from flow cytometric DNA histograms, where it is taken to be the population intermediate between diploid and tetraploid. This measurement has not only shown poor inter- and intra-laboratory reproducibility, but at best gives a result based upon a mixed population of tumor cells, connective tissue cells, inflammatory cells, and endothelial cells. The surgical pathologist knows that what looks grossly to be tumor
may contain only a small fraction of tumor cells and that the only way to characterize a reasonably pure population of tumor cells is by microscopic sorting by an observer, as with image analysis. For these reasons flow cytometric results will not be discussed here.

The first measurement of the S-phase fraction of a human ductal carcinoma of the breast was carried out in 1959, using \textit{in vivo} labeling with tritiated thymidine followed by autoradiography and visual cell sorting.\textsuperscript{7} This was an aggressive tumor with widespread metastases, and yet the S-phase fraction was only 0.14% as compared with 0.36% in a benign serous cystadenoma of ovary and 0.38% in the basal layer of normal human skin.

In 1960 another study, this time using \textit{in vitro} labeling with tritiated thymidine, compared the S-phase fractions of a fibroadenoma of breast, with a ductal carcinoma and its associated non-neoplastic duct epithelium.\textsuperscript{8} Again, it was surprising to find that the S-phase fraction of the cancer population (0.44%) was less than that of either the benign adenoma (1.7%) or the normal duct epithelium (0.65%).

At that time it became evident that malignant transformation did not necessarily result in a speeding up of the mitotic cycle and that the S-phase fraction alone could not be used as a criterion of malignancy. In other words, there is clearly a dissociation of cell doubling time from the capacity to invade and metastasize.

The many measurements of the S-phase fraction since then have shown a wide variation. Measurements within one laboratory, using consistent techniques and visual cell sorting, have shown S-phase fractions with a range of 0.01% to 19%,\textsuperscript{9} values both much greater and much less than that of non-neoplastic duct epithelium. Malignant transformation can be associated with either a speeding up or a slowing down of the mitotic cycle. Accordingly, the S-phase fraction cannot distinguish benign from malignant tissue.

\textbf{The S-Phase Fraction and Clinical Prognosis}

The S-phase fraction is an indicator of growth rate, but not of clinical malignancy. A very slowly growing breast cancer can reveal a frightful capacity for systemic spread. In fact, ductal carcinomas of the breast, even the slowest growing, are often, if not usually, systemic at the time of first diagnosis. It is not unusual for slowly growing metastases to become clinically evident for the first time some 5, 10, or more years after removal of the primary.
The rate of growth of metastases usually determines the length of the recurrence-free interval. This interval can be estimated from the S-phase fraction using Equation 4. Since these calculations are rough approximations, they serve only to illustrate a principle and cannot serve as a clinical guide. In figure 2 are shown three computer generated curves based upon three different values of S-phase fraction, $F_s$, using the following model. The diameters of metastatic tumors are the diameters of spheres consisting entirely of tumor cells, and the number of cells at any given time is calculated from Equation 4. The cell size is based upon a mean cell diameter of 30 microns. The duration of the S-phase ($T_s$) is five hours. Each metastasis begins as a tumor embolus of ten cells ($N_0$). If a tumor mass 2 cm in diameter in a lymph node, bone, liver, or brain will begin to cause clinical signs or symptoms, then S-phase fractions of 0.01, 0.002, and 0.001 will be associated with recurrence-free intervals of 1, 5, and 10 years.

If the S-phase fraction does not change appreciably as the tumor progresses, then it is a sensitive predictor of the growth rate and the recurrence-free interval. But is it a valid criterion for therapeutic decisions? In the “node negative” patient with early metastases of just a few cells, is adjuvant therapy not just as important for a slow growing metastasis as for a fast growing metastasis, particularly in a young person?

Implications for Cancer Cell Biology

It is often assumed that the rapid growth rate of malignant neoplasms is due to an accelerated mitotic cycle, deregulated perhaps by an oncogene product. However, measurements of the S-phase fraction of tumors have shown that cancer cells have abnormal mitotic cycles that may be either longer or shorter than those of their non-neoplastic counterparts. One tends to assume that the rapid growth of a tumor requires that it have a shortened mitotic cycle. In fact, a maturation defect can cause exponential tumor growth even with a prolonged mitotic cycle time.

An accelerated mitotic cycle should not be viewed as a necessary attribute of the cancer cell. Most cancer cells probably have much longer mitotic cycle times than their normal stem cell precursors. Intestinal crypt cells of the mouse divide about once every 19 hours. An intestinal tumor with a similar mitotic cycle time would more than double in size every day! An abnormal mitotic cycle time in a cancer cell appears to be only one feature of a general failure of maturation which includes a failure to control its capacity to invade and metastasize as well as its failure to develop a variety of normal cell functions. Exponential population growth owing to maturation failure but without an accelerated mitotic cycle is shown schematically in figure 3.

Normal stem cell populations in the body are maintained in a steady state by the fact that, on average, one of the two daughters of each stem cell division enters a maturing sequence, whereas the other remains an immature stem cell,
ON THE CLINICAL SIGNIFICANCE OF THE S-PHASE FRACTIONS OF TUMORS

Figure 3. Effect of cell maturation on population size. Although all proliferating cells have the same mitotic cycle time, the lower population fails to mature and therefore grows exponentially, whereas the upper population remains in a steady state. Even if the lower population had a much longer mitotic cycle time, and therefore a smaller S-phase fraction, than the maturing population, it would still grow exponentially to produce a tumor.

ready to divide again. If any more than half of the stem cell progeny fail to mature, then the population will grow exponentially, producing a tumor, regardless of the duration of the mitotic cycle and regardless of the S-phase fraction.

It would seem wise to place less importance on the cellular control of mitosis and more on the control of maturation. Therapy directed toward promoting maturation of cancer cells has been successful in the case of promyelocytic leukemia and is showing promise in other tumors.10

References