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Time Dimension in Histopathology

G. Zajicek
H. H. Humphrey Center for Experimental Medicine and Cancer Research Hebrew University-Hadassah Medical School, Jerusalem, Israel

SUMMARY

In the liver acinus hepatocytes and littoral cells stream from the portal tract toward the terminal hepatic vein. Their average displacement trajectory was denominated as tissue radius. Since cells advance on the tissue radius in one direction, the farther a cell is the older it is, and cell age may be estimated from its position. This property is regarded as time dimension of a tissue. Since distance may be estimated by image cytometry, age determination may be incorporated in the image cytometer software. Distance may be expressed in two types of units: 1. metric e.g. μm, and 2. cell location or the number of cells separating a given cell from tissue origin. Cell age may also be expressed by two units: chronological and biological. Chronological age may be derived from the cell's displacement velocity. A hepatocyte advances daily 2 μm so that a cell at 100 μm distance is 50 days old. Biological age is defined as the cell's location on the radius. Cell location may serve as biological age unit since cells ranked according to their location are also ranked by their ages. The average acinus radius is 22 location long so that in biological age units, maximal hepatocyte life span is 22 locations. One may even say that a cell at location 5 is 2 biological units older than a cell at location 1. In order to estimate chronological age the cell has to be followed for a period of time, while biological age may be read off once from the section. There is yet another advantage for utilizing cell location as unit. Following a mitosis one of the daughter cells replaces the parent while the second is displaced by one location. If expressing hepatocyte displacement velocity in location units, hepatocyte velocity equals cell production rate, since one newly formed cell is associated with the displacement of all distal cells by one location. In summary if mapping hepatocytes on the tissue radius cell location may serve for estimating biological cell age and cell production rate. Both can be read off from a single cytometer scan provided it has been made along the tissue radius.

Streaming Tissue theory

Cell division is linked with cell displacement. Following a mitotic division, one of the progeny replaces the dividing parent cell while the other is displaced into an adjacent location. Whenever a cell divides one of its progeny has to move. This is regarded here as a fundamental relationship of Cell Proliferation Kinetics that links cell production and displacement. One cell division results in a displacement of a cell by one location. In epidermis, proliferating cells are in the basal layer. Following a division of a basal cell, one daughter cell generally replaces the dividing parent remaining a basal cell. The other is displaced into the first supra-basal location. Yet in order to enter the new location, all cells above it have to move outward by one location. A mitotic division results in a displacement of all peripheral cells by one location. Once leaving the basal layer, keratinocytes attach to each other by desmosomes and advance together. Apparently cells are neither pushed nor pulled in a mechanical sense, this is why we regard this

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movement as streaming. Cell streaming is nourished by basal progenitors. The more basal cells divide, the faster the stream of supra-basal cells. This type of streaming is proliferation dependent and has to be distinguished from other forms of streaming, e.g., intravascular erythrocytes, which are propulsed by pumping heart, and their streaming does not depend on cell proliferation. Cell streaming occurs in rapidly proliferating epithelia, e.g., crypt-villus, cervix, and in slowly dividing tissues, e.g. liver, adrenal cortex and salivary glands[1–5].

Streaming Liver

In the liver acinus hepatocytes stream from portal tract to terminal hepatic vein, which was demonstrated by us by labelling hepatocytes with tritiated thymidine and following their progress through the acinus[3]. At each time interval the distance of labelled cells from the portal tract was measured. Figure 1 depicts two frequency distributions of hepatocyte distances from portal tract rim, 1 h and 120 days after labelling. Initially most labelled hepatocytes are not farther than 200 μm from portal tract rim. In the following days hepatocytes stream through the acinus toward the terminal hepatic vein and the frequency distribution shifts away from the portal tract. Tritiated thymidine is incorporated by hepatocyte progenitors. The frequency distribution of labelled hepatocyte distances observed one hour after labelling, delineated the acinus region in which cells proliferate, known as P-compartment ("P" for proliferation). The rest of the acinus, devoid of labelled hepatocytes is called the Q-compartment ("Q" for quiescent). Figure 1 depicts that hepatocytes are formed in the P-compartment and stream into the Q-compartment which is illustrated schematically in Fig. 2. The average trajectory of the stream is called acinus-radius. As hepatocytes stream, they age and differentiate. The more remote a cell is from the portal tract, the older and more differentiated it is. Since hepatocyte streaming depends on progenitor cell turnover, aging and differentiation depend on cell turnover as well. This interesting relationship will now be evaluated.

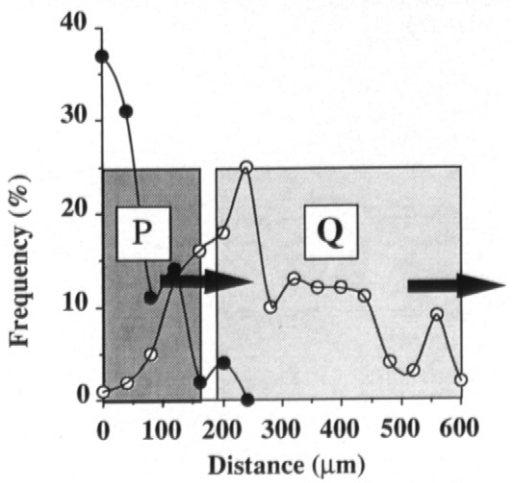

Fig. 1. Frequency distribution of labelled hepatocyte distances from portal tract, one hour (black circles) and 120 days (empty circles) after labelling with tritiated thymidine. The site of labelled cells marks the extend of the progenitor (P) compartment. The rest of the acinus marks the quiescent (Q) compartment.

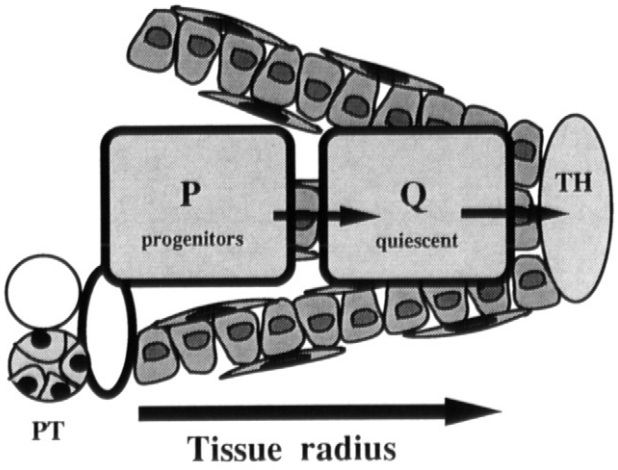

Fig. 2. Schematic representation of the hepatic acinus unit and its tissue radius. Cells stream, differentiate and age along the acinus-radius. TH-terminal hepatic vein. PT-portal tract.

Chronological and Biological Age

Figure 3 depicts the acinus-radius, as hepatocyte chord extending from portal tract to terminal hepatic vein. Cells on the radius are numbered starting at portal tract rim. The average length of the acinus-radius is 22 cell locations. Since hepatocytes stream toward the terminal hepatic vein, the farther a cell is from the portal tract, the older it is. A

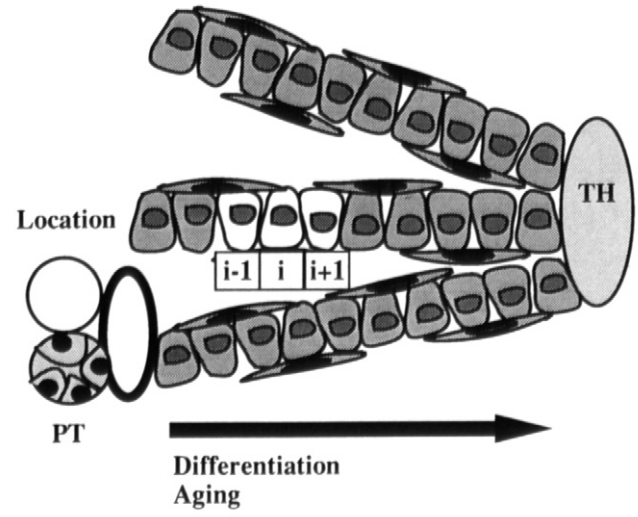

Fig. 3. Schematic representation of three hepatocyte states on the tissue radius. TH-terminal hepatic vein. PT-portal tract.

cell at location i is older than its proximal neighbor at location $i-1$ and younger than its distal neighbor at location $i+1$. Generally a cell at $i+1$ is the immediate future of a cell at i and its proximal neighbor at location $i-1$ is its immediate past. By counting locations one can roughly estimate hepatocyte age. Although this method does not provide the cell's exact age, hepatocytes may be ranked according to their relative ages. In other words we can say that a cell at location $i+1$ is older than a cell at i, even if we do not known their exact ages.

We may thus distinguish between two age measures: chronological and biological. The first is given in usual time units like minutes and hours. Biological age is expressed in cell location units. For instance, a hepatocyte at location 5 is three location units older than a cell at location 2. The advantage of biological age that it may be read off any histopathological section, while chronological age estimates are more difficult and time consuming. In order to estimate the chronological age of a hepatocyte at location 2, the animal has to be labelled with triated thymidine, labelled hepatocytes have to be followed with time. The average hepatocyte diameter is 20 μm, and hepatocyte velocity 2 μm/day[3]. At this velocity a hepatocyte traverses one cell location in 10 days, and the chronological age of a cell at location 2 is 20 days.

It is striking that in pathology chronological cell age is not so important. One is more interested in relative ages of cells in a tissue, or in its overall appearance. The Streaming Tissue theory provides a conceptual tool for estimating relative biological age. One has first to outline the tissue radius along which cells stream. On this radius, cell locations are proportional to biological age and may serve as time units. Once demonstrating that hepatocytes stream across the acinus it becomes obvious that cells at zone-1 are younger than cells in zone-3 and that their enzymatic make up is that of young hepatocytes. Since hepatocytes in zone-1 do gluconeogenesis, and zone-3 cells do glycolysis, one may suspend hepatocytes and determine their age by virtue of their enzymatic make up, or choose any age dependent (or location-dependent) histochemical marker. This property of the tissue is regarded here as its time dimension and may be estimated during a routine inspection of histopathological sections.

Fundamental Relationship of Cell Kinetics

Biological time provides a link between three seemingly unrelated phenomena, cell turnover, aging and differentiation. We have seen above that one mitosis at portal tract rim is associated with displacement of hepatocytes on the acinus-radius by one location. Since the same cell location also serves as biological time unit, we may say that the same mitosis is associated with aging of peripheral cells by one location unit. For instance, in order for a cell to age 3 location units, 3 hepatocytes have to divide. Generally in order for a cell to age by i location units, i cell divisions are necessary. Cell turnover is thus linked with cell streaming and cell aging, and both are expressed by the same unit. Since biological age is actually a form of differentiation, cell turnover and differentiation are linked.

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Prof. G. Zajicek, Medical Computer, P.O. Box 1172, Jerusalem 91010, Israel