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CELL DIFFERENTATION, AGING AND CANCER: THE POSSIBLE ROLES OF SUPEROXIDE AND SUPEROXIDE DISMUTASES

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ABSTRACT

A unified theory of cell differentiation, aging, and cancer is discussed. All cells are hypothesized to originate from stem cells. These stem cells mature as they divide and eventually reach a fully differentiated cell, which cannot divide. Aging is caused by the loss of stem cells, either due to cell death or terminal differentiation, and by eventual death of fully differentiated cells. Both loss of stem cells and death are brought about by oxygen radicals.

The cancer phenotype is caused by an inability of a stem cell to differentiate fully under the local environmental conditions. Because the cancer cell cannot differentiate, it never loses its potential for growth. The block in differentiation of cancer cells is caused by a relative lack of radical scavengers, particularly manganese superoxide dismutase, coupled with production of radicals, especially superoxide. The high reactivity of these radicals leads to changes in key subcellular structures and prevents the cell from attaining the organization needed for cell differentiation to occur.

Key Words: Cancer, Aging, Differentiation, Superoxide, Superoxide Dismutase

INTRODUCTION

All forms of life must be understood in an evolutionary context. This includes the cancer cell as well as any normal cell. Life arose on the earth in a reducing atmosphere free of molecular oxygen. As a consequence, the earliest forms of life were simple organisms whose main function was to replicate. These organisms were unicellular and did not have the capacity to carry out the specialized functions characteristic of higher organisms. This situation changed drastically with the introduction of oxygen into the atmosphere. This high energy source apparently allowed the development of more complex life. It is only in these higher organisms that we see the development of the disease known as cancer. Thus, there must be some connection between oxygen, evolution, and cancer; this idea is not new and has been previously proposed by Szent-Gyorgyi (1).

The development of any higher organism from single cell to adult essentially mimics the process of evolution. Higher organisms start out as single cells which derive their energy from glycolysis, the simple biochemical pathway used by all simple organisms. As they develop they of course become multicellular and derive more and more of their energy from oxygen by the process known as respiration. The reason why higher organism development mimics evolution is simple: new organisms arose by building on existing cellular machinery, not by totally replacing the old organism. Higher organisms evolved by adding biochemical pathways to those already existing; of course, as they evolved, some pathways were also deleted. In this paper, we are proposing that the cancer cell is a cell that is arrested in its development so that it resembles a more primitive type of cell. The difference between transformed and normal cells is the inability to differentiate fully under the local environmental conditions.

Linked with the concept of evolution is the idea of mortality of organisms. Unicellular organisms are basically immortal: they can divide for an unlimited number of generations - the total number of possible cell divisions in a population is infinite. (This does not imply that each individual unicellular organism is immortal.) In contrast, multicellular organisms have only a finite lifespan: each individual cell of the organism can divide only for a finite number of generations - the total number of possible cell divisions in a population is finite. The cancer cell is like the primitive cell in that it can apparently divide for an infinite number of generations.

Why can a unicellular organism produce an infinite number of generations while the cells of a higher organism produce only a finite number of generations? The answer to this again is given by evolution. The primary goal of any living organism must be to continue the population. In order for the population to meet the needs of a changing environment it is necessary that new organisms be introduced into the environment (with

new genes). Thus, reproduction is a necessity. In the unicellular organism, the population must have an infinite capacity to pass on its DNA. If a population could divide only for a certain number of generations, the population would die out. Multicellular organisms also must have an infinite capacity for reproduction. If man could only reproduce for 40 generations, mankind would have long ago died out. Thus, all organisms have an infinite or near infinite capacity to pass on their DNA to the next generation of organisms. However, each individual cell in a higher organism has only a finite capacity to pass on its DNA. This is because each environmental niche will hold only a finite number of organisms; this dictates that death of organisms occurs. A unicellular organism consists of a single cell, while a multicellular organism consists of several or many cells. If the cells in higher organisms had an infinite capacity to pass on their DNA to the next generation of cells, higher organisms would only rarely die (due to accident, etc.). Thus, in order for natural selection to occur, there must be reproduction, death and a limited number of cell generations. A population of cells from a higher organism will cease dividing when a cumulative total number of mitotic events have occurred. Aging occurs in higher organisms because the proliferative potential of each cell is lost, not because the lifespan of any one cell is naturally shortened. In this context we must be careful to note the difference between organismal aging and cellular aging. Organismal aging is that process whereby multicellular organisms naturally die. We believe organismal aging is caused by loss of proliferative potential of its cells plus death of its fully differentiated cells - cellular aging.

One of the best ways to insure survival of higher organisms is to link loss of proliferative capacity with an environmental factor necessary for life. We believe that factor is oxygen. We hypothesize that the cause of aging is the loss of proliferative potential of individual cells because of changes caused from oxygen metabolites, as well as death of fully differentiated cells because of environmental factors including oxygen. The cancer cell is immortal because it does not fully differentiate and thus never loses its proliferative potential.

EXPERIMENTAL DATA ON CELL DIFFERENTIATION AND AGING

Most of the work relating aging and cell differentiation has been discussed by Bell *et al.* (2). In this paper, one further aspect must be discussed - the relationship between aging and superoxide dismutase (SOD). Fridovich first proposed that damage caused by oxygen radicals was responsible for aging (3). Since that time, many studies have been done on the superoxide dismutase levels of tissues of different age with ambiguous results: some have found a decline of SOD activity with age and some have not (4,5,6). These results have recently been clarified by Tolmasoff *et al.* (7). They measured SOD specific activity in cytoplasmic fractions of liver, brain, and heart of 2 rodent and 12 primate species. These species have lifespans ranging from 3.5 to 95 years. Liver, brain, and heart had similar

specific activity levels for a given species, but for different species, levels varied up to threefold. No correlation was found in the level of SOD with lifespan. However, the ratio of the SOD specific activity to specific metabolic rate of the tissue or of the whole adult organism was found to increase with a high linear correlation with increasing lifespan of the species. Specific metabolic rate is an indirect measure of O_2^- production. Thus, this study shows that it is the SOD/O_2^- ratio that is important in aging. These results suggest that increased SOD protection against by-products of oxygen metabolism was necessary for an increased lifespan to evolve.

With regard to SOD and cell differentiation, it has been shown that SOD increases with donor age in skin fibroblasts; cells of low population doubling time also exhibited low SOD activity (8). Moreover, SOD activity has been shown to increase in rat liver during development from fetus to adult (9). This suggests that as a cell matures, some form of SOD activity increases. This is also suggested by the Morris hepatoma data, which will be discussed later. Moreover, it also appears that superoxide production increases as a cell matures. This is suggested because fetal and stem cells derive most of their energy from glycolysis (10,11). As the cell matures, more and more of its energy is derived from respiration, thus producing more O_2^-. This result is also demonstrated by the Morris hepatomas, as will be discussed later.

OXYGEN DEPENDENT INHIBITION OF GROWTH

Two investigators have shown that oxygen inhibits growth of populations of normal cells but not populations of transformed cells (12,13). Goetz has shown that hamster embryo cells die out in the presence of high concentrations of O_2, while cells transformed by chemical carcinogens continue to proliferate in O_2 (12). Mitchell et al. have shown that in an incubator enriched with oxygen, normal fibroblasts are maintained without proliferation, while Rous sarcoma virus-infected fibroblasts continue to proliferate (13).

These results are ambiguous at present because it is not known if oxygen inhibits normal cell proliferation or increases cell death. It seems most likely that oxygen does both. We have recently shown that oxygen radicals can kill a nondividing, fully differentiated cell population (14). However, it took a very large amount of superoxide to kill these cells, suggesting that either rapidly dividing cells must be more susceptible to oxygen induced killing than fully differentiated, nondividing cells or else that dividing cells cease to proliferate in the presence of oxygen.

FREE RADICALS AND AGING

Munkres and Minssen have summarized the evidence for the free radical theory of aging (15). In addition, they have provided valuable new data in support of this theory by their study of a recessive mutant of Neurospora crassa, called natural death (nd), which is characterized by a decreasing clonal growth potential under all nutritional conditions

of growth and the irreversible cessation of growth. Antioxidants such as nordidroguaiaretic acid, 3,4,di-tert-butyl-4-hydroxybenzyl alcohol, D-α-tocopherol acetate, L-ascorbic acid, and sodium selenite increase significantly the lifespan of this mutant. Moreover, superoxide dismutase, glutathione reductase, and glutathionine peroxidase were increased two- to fourfold in the mutant as compared to the wild type Neurospora, suggesting an increased flux of oxygen radicals in the mutant cells. An inositol auxotroph of Neurospora crassa also aged at a high rate and showed large amounts of lipid peroxidation (16). The aging and lipid peroxidation could again be alleviated by antioxidants. The authors suggest that oxygen free radical reactions and associated lipid peroxidatior contribute to the senescence and death of these organisms.

MODEL FOR CELL DIFFERENTIATION AND AGING

Our model for cell differentiation is patterned after that of Pierce (17), while our model for aging is essentially that of Bell et al. (2). This model is shown in Figure 1:

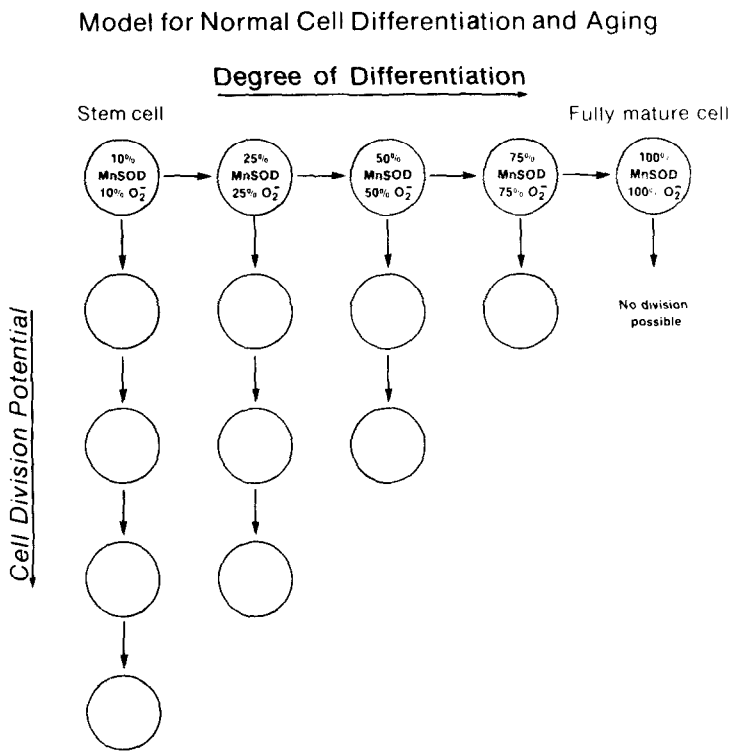

Figure 1. Model for normal cell differentiation and aging. The numbers in the figure represent approximate percentage of normal differentiated cell activity. Mn SOD is the manganese containing superoxide dismutase and O_2^- represents superoxide radical.

The cells of all multicellular organisms (adult or embryo) are hypothesized to start with stem cells. These stem cells give rise to all other cells by the process of differentiation. The stem cell will divide a number of times, either producing a cell of the same state of differentiation or one that will go on to differentiate further. Obviously, some of the cells must stay stem cells after division, or else the pool of stem cells of the organism would soon diminish. The most simplistic view is that in each division one cell remains a stem cell while the other goes on to differentiate. As the cell differentiates, it loses some of its potential to further divide. The fully differentiated cell has little or no potential for cell division. This differentiated growth-arrested cell has only one fate: death. Death may be caused either by environmental factors or else a natural process called cellular aging. We believe that aging (in-vivo and in-vitro) is caused both by loss of stem cells by cell differentiation with inhibition of cell division and/or death, and by the death of fully differentiated cells. Lack of division potential means that the cells cannot replace themselves as they die and this must eventually lead to death of the organism. Bell *et al.* do not believe that cells themselves age. This seems an extreme view.

This theory of aging is best understood by looking at a few examples. In the mammalian intestine, the stem cells are crypt cells found at the base of the villi. After cell division, one of the progeny remains in the crypt as an undifferentiated stem cell, whereas the other cell migrates along the surface of the villus and differentiates into one of the highly specialized cells that performs the function of mature gastrointestinal epithelium (17). These differentiated cells do not undergo further division. The life of such a cell is approximately 4 days and after this time, the cells are sloughed from the tip of the villus into the fecal stream. Normally, cell production equals cell loss - a dynamic equilibruim. We believe aging in such a system occurs because of loss of stem cells - either due to terminal differentiation or stem cell death. Terminal differentiation could cause stem cell loss if both cells after a division go on to differentiate, rather than one cell remaining a stem cell.

An opposite extreme from this example is the case of human brain cells. Neuronal cells of the brain do not divide after a person reaches 2 years of age. In this case, aging in the brain must be caused by death of fully differentiated cells. Stem cells are not involved here, but the fact that terminally differentiated cells cannot divide again is what causes senescence.

In some cases, both stem cells and fully differentiated cells are involved. An example here is the case of in-vitro mammalian fibroblasts. Numerous experiments have shown that these cells will divide for a certain number of generations - i.e., when a cumulative number of mitoses have occurred. The results are easily understood when it is realized that these cultures consist essentially of two populations - a dividing population (i.e., stem cells) and a non-dividing, differentiated population. When the dividing population is lost - either due to terminal differentiation or due to death - only differentiated cells, which cannot replace themselves, remain. Aging in this case is caused both by loss of the dividing cell and death of fully differentiated cells.

We have hypothesized that organismal aging is caused by:

1. loss of proliferative potential because of differentiation and/or death of stem cells;
2. death of fully differentiated cells due to environmental stress resulting in slow cellular aging.

Evolution can insure that both of these happen by coupling aging with an essential ingredient for life. We believe this ingredient is oxygen. We hypothesize that oxygen causes loss of proliferative potential of stem cells either by inducing differentiation of all stem cells or else by causing death of stem cells. There is some evidence that the latter is what is important. Stem cells appear to be especially vulnerable to oxygen radicals because of low levels of Mn SOD. Petkau has shown that bone marrow stem cells are more vulnerable to radiation (a known producer of oxygen free radicals) than other more fully differentiated blood cells (18). We also hypothesize that once cells are fully differentiated, oxygen radicals cause death because some will escape all forms of SOD and cause accumulated damage.

In all stages of differentiation, changes in the cell are not caused by any loss of superoxide dismutase. This is because both O_2^- and SOD increase as the cell differentiates, implying there is enough SOD to handle the O_2^- flux. Moreover, most of the evidence shows the SOD activity does not decrease with age in mature organisms. Thus changes involved with aging occur because some radicals escape the defense mechanisms such as superoxide dismutase. This probably occurs because radicals are so reactive.

The evidence for most of this model has been presented. However, what is the evidence that oxygen is the precipitating species in organism aging? First of all, as stated earlier, it has been shown that high levels of oxygen stop the proliferation of populations of normal mammalian cells (12,13). This cessation of proliferation must be for one of three reasons:

1. they are caused to differentiate and hence stop cell division;
2. they cease to divide even without cell differentiation;
3. oxygen kills normal cells under these conditions.

With the present data, it is impossible to differentiate between these three possibilities. The most likely explanation of this data is that oxygen radicals affect the dividing population of in-vitro cultures causing this population to be lost through differentiation or death.

Moreover, the above model explains why SOD/metabolic rate ratios increase with lifespan in primate species (7). Higher SOD/O_2^- ratios lead to better protection against superoxide. Better protection may protect the stem cell population, as well as protect the fully differentiated cells from oxygen metabolites.

Lastly, the above model explains why SOD levels are high in the aging mutant of Neurospora crassa (15). Superoxide levels must be higher in this mutant so that higher levels of SOD are needed to protect against

this radical. Radicals that escape this SOD lead to lipid peroxidation and accumulated damage which accelerage aging in the mutant.

EXPERIMENTAL DATA ON CANCER

We have based our theory of cancer on the following experimental observations. Many studies have been made on the superoxide dismutase activities of normal and malignant cells. A typical pattern has emerged from these studies. Cancer cells have in general lowered amounts of both copper-zinc containing superoxide dismutase (CuZn SOD) and manganese containing superoxide dismutase (Mn SOD) when compared to their normal cell counterparts. Exceptions have been found to this pattern of low CuZn SOD, but no exceptions have been found in the case of Mn SOD. Mn SOD activity has been found to be greatly reduced in spontaneous, transplanted, virally-induced, chemically-induced, in-vivo and in-vitro tumors. This work has been the subject of a recent review (19).

We have questioned the significance of these enzyme changes because the cancer cell exhibits numerous enzyme alterations. Why is SOD any more important than any other enzyme? The answer to this question may be that SOD is fundamentally different from most other enzymes in that it is a protective enzyme. Indeed, Fridovich has provided conclusive evidence that this enzyme is necessary for life in all oxygen metabolizing cells (20). We feel that loss of SOD enzymatic activity in the cancer cell will lead to changes in key subcellular structures because of the presence of oxygen derived radicals.

However, SOD is a protective enzyme only if its only substrate, superoxide radical (O_2^-), is present in the cancer cell. If O_2^- is not produced in the cancer cell, then loss of Mn SOD should not lead to any harmful effects. From these considerations, it can be seen that in order to establish that loss of Mn SOD activity is important in malignancy, it is also necessary to show the production of superoxide in tumor cells. We and others have shown that tumor cell mitochondria have the capacity to produce superoxide (21,22,23).

Thus, diminished amounts of Mn SOD activity coupled with superoxide production in the cancer cell appears to be a general characteristic of tumor cells. The net result is an increased flux of superoxide in the cancer cell. Superoxide itself can react with various cellular compounds, as discussed in our review article (19). Superoxide itself is not especially reactive and may not cause any significant cellular changes. However, the presence of superoxide can result in the formation of more reactive species, such as hydroxyl radical, singlet oxygen, hydroperoxides, etc. These species can in turn damage key subcellular structures or cause a shift in biochemistry to prevent or compensate for such damage. It should be emphasized at this point that it is not known how early in the development of the cancer cell phenotype the loss of Mn SOD occurs.

Many steps may need to occur before Mn SOD is diminished and, thus, probably not all of the cancer cell phenotype is caused by loss of Mn SOD.

MODEL FOR CANCER

Our model for cancer is shown in Figure 2:

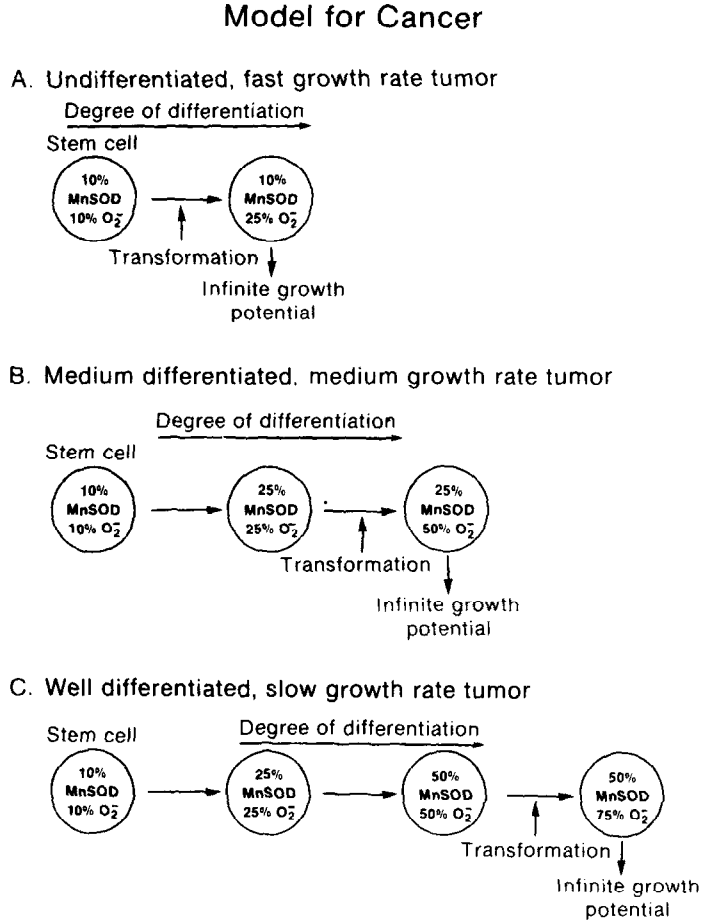

Figure 2. Model for cancer. The numbers in the figure represent approximate percentage of normal differentiated cell activity.

This model has again been suggested by Pierce (17). We believe cancer is caused by inability of the cancer cell to fully differentiate under local environmental conditions. Because of this lack of differentiation, a cancer cell does not lose its ability to proliferate as does a normal cell.

This inability to differentiate must involve Mn SOD. CuZn SOD may also be involved in some cases; it cannot always be involved because this enzyme is high in some tumors (19) and decreases with cell differentiation in the mucosal cells of the large intestine (24). Again, our model says that cancer cells are derived from stem cells. Some time in the process of maturation, transformation occurs and differentiation is halted. If transformation occurs early in the cell differentiation process, an undifferentiated, fast growing tumors arises. If it occurs later, a well differentiated, slow growing tumor arises. Transformation cannot occur in the fully differentiated cell because this cell has lost the capacity to proliferate. This inability to differentiate is caused by diminished Mn SOD coupled with normal O_2^- levels; the altered Mn SOD/O_2^- ratio is the true reason for the inability to differentiate. The altered ratio causes changes in key subcellular structures. Thus, cancer is caused by:

1. As a normal stem cell differentiates, transformation occurs. Transformation occurs by a change in nuclear DNA (mutation) or its expression (nonmutational or reprogramming). This change in the expression of DNA prevents the increase in Mn SOD normally seen as the cells mature. Superoxide levels increase as normal cells mature and are not decreased after transformation.

2. This failure to increase Mn SOD along with increased O_2^- levels results in a cell with increased fluxes of O_2^-. Superoxide itself, or radicals derived from it, cause damage to key subcellular structures or cause a shift in biochemistry to avoid or compensate for such damage. These changes are responsible for many of the phenotypic properties of the cancer cell - including the inability to differentiate and lose cell division potential.

3. At the same time or at some other time, changes occur which affect the sensitivity of the cancer cell to oxygen. The cancer cell either is not susceptible to inhibition of cell proliferation by oxygen or it is not susceptible to oxygen induced killing. The latter must be at least partly true because the cancer cell has no Mn SOD. According to current theory, if it were a normal cell, it would necessarily die.

These points are discussed below. One should note that our model is similar to that proposed by Szent-Gyorgyi (1), except that he does not identify the radicals involved.

Because of this excess of superoxide production (in comparison to the amount of SOD activity), we believe the cancer cell resembles some of the first eukaryotic cells. These cells must have started to use oxygen and thus produce superoxide before superoxide dismutase appeared on the scene. Because of the excess of O_2^- in these early cells, differentiation and specialization could not occur because these depend on organization. Because of the high chemical reactivities of oxygen radicals and species derived from them, this organization could not be attained. Thus, true multicellular organisms were an impossibility. The cancer cell is like

this primitive cell: it has an excess of O_2^- over SOD. Thus, organization and structure cannot be maintained. This relationship between organization (differentiation) and cancer has been discussed by Szent-Gyorgyi (1).

Yamanaka and Deamer have performed experiments that are in agreement with this hypothesis (25). In-vitro trypsinization of normal confluent WI-38 lung fibroblasts caused loss of Mn SOD. It has been shown by several groups that such trypsinized cells, if they are part of a confluent, non-dividing monolayer, escape from growth control and most of the cells go through one round of cell division. Thus, normal cells, if they lose their Mn SOD, also divide, possibly because of the excess superoxide present.

TARGET CELL

We believe the target cell for malignant transformation is the stem cell of the various cell renewal systems. Once a cell is fully differentiated it cannot be transformed. This is of course not a new hypothesis, but was first proposed by Pierce (17). Our work with SOD supports his hypothesis. We have examined 1,2-dimethylhydrazine (DMH) induced large bowel tumors in the rat (24). These tumors had nearly equal levels of CuZn SOD when compared to the crypt cells of the large intestine, the cell widely thought to be the stem cell of the large intestine. In contrast, villus cells, mature differentiated cells of the intestinal epithelium, contained vastly lowered levels of CuZn SOD. Tumor cells had lower Mn SOD activities than either crypt or villus cells. The overall SOD activity of the tumor cell was most similar to the crypt cell, suggesting this was the cell which was transformed.

CELL DIFFERENTIATION AND CANCER

Our model for cancer involves the differentiation of normal stem cells into mature differentiated cells. Malignant transformation occurs somewhere in the timespan of cell differentiation and acts on the stem cell.

Normal cell differentiation is influenced by the oxygen metabolism of the cell. Stem cells derive most of their energy by glycolysis and contain few mitochondria (10,11). As the stem cell progresses through differentiation, more and more of its energy is derived from respiration in the mitochondria and less and less through glycolysis. This means that as the cell matures, increased fluxes of superoxide are produced in the normal cell due to electron leakage from the mitochondrial electron transport chain. These higher levels of O_2^- lead to higher levels of Mn SOD.

The tumor cell evolves because of a change in DNA expression which halts this progression to maturity. The tumor cell can arise in any cell on the way to maturity except the fully differentiated cell. Thus, if malignant transformation occurs early in the stem cell cycle, a fast growing, undifferentiated tumor will arise. If it occurs late in the development of the stem cell, a slow growing, well differentiated tumor will arise. The concept was introduced by Pierce (17) largely because of his studies of the Morris hepatomas. These tumors show a spectrum of phenotypic properties from undifferentiated fast growing tumors to well differentiated slow growing tumors. The variation in phenotypic properties is part of the basis of Pierce's hypothesis.

Our studies with the Morris hepatomas are consistent with this view (23). Furthermore, they led to our proposed model of cancer. We found that submitochondrial particles from the Morris hepatomas produced O_2^- at only one third the rate of normal liver submitochondrial particles. We believe that fast growing Morris hepatomas are derived from stem cells very early in the maturation of this cell. Slow growing Morris hepatoma submitochondrial particles had the same rate of O_2^- production as normal liver. This is to be expected since this hepatoma is derived from a nearly completely differentiated stem cell.

Mn SOD levels were also measured in these hepatomas. It was found that total Mn SOD is reduced in all the Morris hepatomas compared to normal liver (23). Even the slow growing Morris hepatoma had less than normal levels (60%) of Mn SOD, even though it had normal levels of O_2^- production. We conclude from this that the cancer cell upon being transformed is halted in its normal process of cell differentiation. In other words, part of the genetic program, that part that allows Mn SOD activity to increase, is blocked. This blockage leads to an increased number of oxygen induced radicals in the cell and this accounts for part of the cancer cell phenotype.

If this hypothesis is true, we should be able to cause certain of the properties of the cancer cell to revert to normal by adding back the lost SOD activity. In the two experiments performed thus far, we have had great success with this approach. Native SOD, because of its lack of penetrability into the cell, was not expected and did not show any great effects. However, copper coordination compounds, which have repeatedly been shown to have SOD activity (26,27), penetrate into the cell, and cause differentiation of in-vitro tumor cells. Thus, 50 μg/ml of Cu(II)(3,5-diisopropyl salicylate)$_2$ caused morphological differentiation of 75% of murine neuroblastoma cells in-vitro (28). Large inhibition of cell division was also noted (28). We have also noted increases in animal survival (~70%) and decreases in cell growth (~30%) of Ehrlich ascites tumor cells in-vivo treated with 18 injections of copper coordination compound (0.5 mg per injection) (29). These effects may also be due to inhibition of cell proliferation and induced differentiation. Thus, added SOD activity seems to be able to overcome the blockage of cell differentiation and allow expression of the normal cell phenotype. This suggests that the blockage of cell differentiation is not irreversible. This had previously been shown in other systems with other agents (19).

LOCALIZATION OF Mn SOD AND O_2^-

We have presented a general model for cancer, but have so far not discussed the precise cellular localization of the superoxide and superoxide dismutase involved. Mn SOD has been thought until recently to be localized exclusively in the mitochondrial matrix. This fact is hard to reconcile with current theory that states that cancer is caused by nuclear changes. Two recent studies have reported the existence of extramitochondrial Mn SOD. McCord et al. have shown that in normal human liver, Mn SOD is also found in the nucleus and that as much as 50% of the Mn SOD is extramitochondrial (30). Mazeaud et al. have shown that carp red blood cells, which do not contain mitochondria but do contain nuclei, have Mn SOD, thus implicating that this enzyme is found in the nucleus (31). Our results with the Morris hepatomas also indicate that Mn SOD is not located exclusively in the mitochondria (23). We found that Mn SOD was lowered in all the Morris hepatomas when compared to normal liver, but mitochondrial Mn SOD was the same in the slow growing Morris hepatoma as in normal liver (23). This, plus various other experiments, indicated that organelle bound Mn SOD other than in the mitochondria was what was reduced in all tumors.

If our conjecture that Mn SOD is found in the nucleus is correct, there must be some reason for its presence. It has recently been shown that isolated Ehrlich ascites tumor cell nuclei produce O_2^- (32); thus, SOD appears to be needed to protect against this radical in the nucleus.

The superoxide indicated in our model could come from any place in the cell which is affected by Mn SOD. Both the nucleus and the mitochondria have been shown to produce O_2^-, with approximately 75% of the O_2^- produced from the latter location (33).

LACK OF SUSCEPTIBILITY TO OXYGEN INDUCED RADICALS

Two experiments have been performed that show that at normal oxygen concentrations dividing normal cells increase in number, but at high concentrations of oxygen they are prevented from doing so. However, it is not known why oxygen inhibits the increase in cell number. Is it because there is a halt in cell division or because more normal cells are killed? Tumor cells are able to proliferate in oxygen indicating that either their cell division is not halted by oxygen or they are not killed by oxygen. This is a subject that must be studied in great detail in the future. The former is an exciting possibility, but there is some evidence that toxicity is important. All of the work being done today assumes that superoxide or its reaction products are very toxic. This is because after exposure to superoxide, rapidly dividing organisms show less growth than unexposed controls. However, most of this data is incomplete because lower cell numbers can be due either to inhibition of cell proliferation or cell death. We have tried to remove some of this

ambiguity by looking at normal, non-dividing cells; alveolar macrophages are killed by oxygen radicals and exogenous SOD protects them (14,34). Moreover, increased levels of endogenous Mn SOD protects these cells from oxygen toxicity (34). Tumor cells, because they do not have Mn SOD, would be expected to die faster in the presence of oxygen. It is possible that tumor cells do die faster in the presence of oxygen but this is offset by an increase in growth rate. However, it has been shown that most tumor cells are much less susceptible to lipid peroxidation than normal cells (35). Since lipid peroxidation is caused by oxygen radicals, this may be an indication that tumor cells are more resistant than normal cells. Furthermore, DDC, a CuZn SOD inhibitor, caused normal cells to be more susceptible to radiation-induced killing (36). We have recently shown that tumor cells were not more susceptible to irradiation after treatment with DDC (37).

Tumor cells could be less susceptible to killing by oxygen induced radicals for two reasons:

1. they may not form the most toxic active oxygen species such as hydroxyl radical from superoxide.

2. they may inherently be insensitive to oxygen derived free radicals because of changes in membranes, etc.

One of the reasons cancer cells are immortal is because they cannot differentiate. As superoxide increases, Mn SOD does not increase concomitantly. Thus, cancer cells can always divide, even in the presence of oxygen. Because of division, they never reach a stage when they are not replaced. Immortality may be attained in the cancer cell both because they never lose the ability to divide and because they are not susceptible to oxygen induced killing. Whichever case is more important, we believe the cancer cell is immortal because it is not susceptible to changes induced by oxygen radicals.

The most likely explanation for this data is the following. Normal cells do not increase in number in the presence of oxygen because their stem cell pool is affected. Either by differentiation or death, the stem cell population is depleted. On the other hand, the tumor cell cannot differentiate. Thus, when it divides, two stem cells are always produced instead of a stem cell and a cell which will later differentiate. Thus, both cell killing and halt in cell division are important. Moreover, if normal stem cells are killed by oxygen, as some of the data indicates, then the cancer cell, which is a stem cell, is abnormal in that it is not affected by oxygen.

SUPEROXIDE AND SOD IN NORMAL AND TUMOR CELL PROLIFERATION

We seemingly have a paradox. One would expect a species such as superoxide to have the same effects in tumor cells as normal cells.

However, increased levels of oxygen and, hence, superoxide, halt normal cell division, whereas an increase in SOD caused cessation of cell division in the tumor cell, and increased levels of O_2^- only makes things worse. This is really not a paradox, but easily explained. O_2^- in the normal cell produces large fluxes of H_2O_2 due to the presence of SOD. This H_2O_2 is responsible for the halt of cell proliferation - either directly or through differentiation. On the other hand, in the tumor cell, a flux of O_2^- would produce only a little H_2O_2 due to nonenzymatic dismutation. However, if SOD is added, the flux of H_2O_2 is increased and the cell can differentiate and cease proliferation. Thus, O_2^- will cause cessation of proliferation in a normal cell. O_2^- in the tumor cell will only make things worse, leading to increased proliferation.

OTHER ANTIOXIDANTS

Thus far, we have only considered SOD in this model. We do not want to leave the impression that it is the only molecule involved. All of the antioxidant compounds must be involved in cancer. For example, catalase is also low in tumors (38,39). Glutathione, vitamin A, vitamin E, and vitamin C are also important parameters to consider. We feel SOD is the most important because it is the first line of defense against oxygen radicals. Other antioxidants are also important as backup systems, in addition to other functions they may have.

For instance, vitamin E, a known antagonist against oxygen-induced lipid peroxidation, has been recently shown to cause differentiation of in-vitro neuroblastoma cells (40). The role of vitamin C in cancer has recently been discussed by Cameron et al. (41). Vitamin C has been shown to act as an SOD (42,43). Szent-Gyorgyi has postulated that methyl glyoxal is responsible for control of cell division (1). We have recently shown that O_2^- reacts with methyl glyoxal (44). Vitamin A and its analogs, free radical or singlet oxygen scavengers, have been shown to slow down the growth of in-vivo and in-vitro tumors (19). We believe combinations of these agents can be used to halt the growth of tumors; combinations are necessary to stop any radical which may leak by any one scavenger and they are necessary to penetrate into all cells and organelles.

CARCINOGENESIS

We have not said what causes malignant transformation and this loss of SOD. This is one of the really untested areas in cancer. It is possible that Mn SOD is not present because it is not induced by O_2^-. Alternatively there may be changes in expression of DNA which lead to loss of Mn SOD directly or indirectly through membrane changes, etc.

CONCLUSIONS

Cell differentiation, aging, and cancer are all closely interrelated. They can all be explained in terms of the principles of evolution. Oxygen is the unifying theme which ties all three together. Thus, oxygen metabolites are responsible for both aging and cancer.

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