ELSEVIER

Mutation Research 400 (1998) 439–446

Fundamental and Molecular Mechanisms of Mutagenesis

Mutagenesis induced by the tumor microenvironment

Jianling Yuan, Peter M. Glazer *

Departments of Therapeutic Radiology, Genetics, and Biology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040, USA

Received 8 January 1998; revised 13 February 1998; accepted 20 February 1998

Abstract

Genomic instability is a commonly observed feature of tumors. Most investigations addressing the mechanism of tumor progression have focused on the genetic factors that may play a role. Growing evidence now suggests that, in addition to these endogenous factors, the exogenous environment within solid tumors may by itself be mutagenic and constitute a significant source of genetic instability. The tumor microenvironment is characterized by regions of fluctuating hypoxia, low pH, and nutrient deprivation. Each of these microenvironmental factors has been shown to cause severe disturbance in cell metabolism and physiology. Both in vivo and in vitro data demonstrate that exposure of tumor cells to adverse conditions can directly cause mutations, contributing to genetic instability. In this review, we will reexamine the current body of evidence on the role of the tumor microenvironment in inducing mutagenesis and consequent tumor progression.

Keywords: Genetic instability; Hypoxia; pH; *supF*

1. Introduction

Genomic instability is a hallmark of malignancy [1]. As cancers develop, they often acquire an increasing number of genetic alterations. At the chromosomal level, aneuploidy, recombination, translocation, large insertions and deletions are commonly observed. At the DNA level, point mutations, such as transitions, transversions and simple frameshifts, have also been recognized. However, the low rate of spontaneous mutation in somatic cells is not sufficient to account for the number of mutations found in malignant cells. Loeb has hence proposed that the expression of a mutator phenotype early in tumor development may be the basis for the high mutation rate associated with many cancers [2]. This notion is supported both by the discovery of human homologs of DNA mismatch repair genes implicated in hereditary forms of colon carcinoma [3] and by other work investigating the role of specific genetic defects that promote genetic instability in mammalian cells [4].

Recently, Richards et al. [5] reported a phenomenon, which they termed a conditional mutator phenotype, in hMSH2-deficient tumor cell lines. These mismatch repair-deficient cells expressed a high mutation rate relative to repair-proficient cells only when they were maintained at high density. Therefore, the growth conditions seemed to have a

* Corresponding author. Department of Therapeutic Radiology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040, USA. Tel.: +1-203-737-2788; fax: +1-203-737-2630; E-mail: peter.glazer@yale.edu

0027-5107/98/$19.00
PII S0027-5107(98)00042-6

strong influence on the behavior of tumor cells. This result is consistent with the hypothesis we and some other labs have proposed, i.e., in addition to genetic factors, the microenvironment of an incipient developing tumor may by itself contribute to genomic instability and mutagenesis, leading to tumor progression and evolution of malignant phenotypes [6,7]. In this perspective, we will describe the characteristics of the tumor microenvironment and discuss mutagenesis within this context.

2. Tumor microenvironment

The tumor microenvironment is characterized by regions of fluctuating hypoxia, low pH, and nutrient deprivation [8,9]. This microenvironmental heterogeneity develops very early in the growth of solid tumors due to inadequacy of blood supply [8]. Although angiogenesis accompanies most growing tumors, the overall tumor vasculature is often disorganized and atypical structurally [10]. In addition, growing tumor cells may invade or compress blood vessels, resulting in further obliteration of the vasculature. Because the diffusion distance of oxygen is exceedingly short, chronic hypoxia results when oxygen tension of a microenvironment drops precipitously with its distance from a functional blood vessel [10]. Even in well vascularized regions of the tumor, fluctuations in microvessel flow rate can produce transient changes in oxygen tension sufficient in magnitude to affect tissue oxygenation [11]. Measurements of hypoxic fractions in a series of primary tumors xenografted onto immune-deficient rodents revealed that significant numbers of hypoxic cells were already present when tumors were still microscopic in size, and that the hypoxic fractions of tumors were independent of the tumor growth rate, the degree of differentiation, or the metastatic potential of the tumor [8,12].

The diffusion distances of glucose and other critical nutrients are similar to that of oxygen [10], and the uptake of glucose is limited by its delivery by the tumor blood flow [13]. Cells lying at a distance from the nearest functional blood vessel may therefore experience nutritional deprivation.

Hypoxic cells depend on glycolysis for metabolic energy [14,15]. Anaerobic glycolysis produces lactic acid and ATP hydrolysis. This, coupled with a diminished ability to remove metabolic waste, results in unphysiologically low extracellular pH, with a median about 0.5 unit below that in normal tissues.

Because the tumor microenvironments cause profound perturbations in metabolism and physiology, it is conceivable that such conditions may either cause increased spontaneous damage to DNA or inhibit DNA repair processes. The ability of cells in these adverse microenvironments to perform the housekeeping and repair functions needed to maintain their genomic integrity may also be compromised.

3. Hypoxia

Among all the microenvironmental factors, hypoxia has been most extensively studied. Much like low glucose levels and heat shock, hypoxia is perceived by cells as a stressful condition. ATP levels decrease rapidly after initiation of hypoxia [16]. G1-phase cell cycle checkpoints are activated [17]. Protein synthesis is decreased and protein degradation is increased, with relatively enhanced synthesis of oxygen-regulated proteins [16,18,19]. Severe, prolonged hypoxia ultimately results in cell death [20,21].

However, transient, intermittent hypoxia due to temporal variations in tumor blood supply is not necessarily lethal to tumor cells. Instead, it may lead to significant genomic alterations. It has been shown that under low oxygen conditions, DNA synthesis is inhibited, due to the suppression of initiation [16,22]. While DNA chain elongation remains unaffected, it may allow for incorporation of possible DNA damage caused directly or indirectly by hypoxia. Cells subjected to repeated hypoxia-reoxygenation have increased intracellular levels of superoxide and other bioactive oxygen radicals that can react with DNA bases [23]. For example, oxidative injury could generate excessive levels of 8-oxoguanine, which has been shown to miscode for A, leading to C:G to A:T transversions [24]. Hypoxia may also damage DNA indirectly by inducing endonuclease activity leading to DNA strand breakage [25,26]. Russo et al. quantified DNA breakage in rat fibroblasts using biotinylated dUTP end-labeling followed by flow cytometric analysis. They found that after a 24-h treatment of hypoxia, while cells remained fully viable, detectable

chromosomal breaks were elevated to about 3-fold above background, paralleling the expression of hypoxia-inducible endonucleases.

The reoxygenation phase following hypoxia also appears to affect cell physiology. Cells recovering from hypoxia have been shown to acquire an increased capacity to initiate DNA replication, resulting in segments of the genome being replicated more than once within a single cell cycle [21,27–29]. Several groups reported that a 20–24-h exposure of cells to hypoxic conditions and subsequent return to normal aerated conditions resulted in the generation of a subset of cells exhibiting >4C DNA content (where C is the content of the haploid genome). Overreplication of DNA, coupled with strand breakage induced during the hypoxic phase, is thought to provide the basis for various forms of chromosomal rearrangements [30]

Gene amplification is the most reported genetic change associated with hypoxia [31]. In 1986, Rice et al. [27] observed that exposure of Chinese hamster ovary cells to transient hypoxia with subsequent recovery in the presence of oxygen led to a marked enhancement in the frequency of methotrexate resistance as a result of the dihydrofolate reductase gene amplification. A 24-h exposure to hypoxia increased the drug resistance by 10-fold, and virtually all cells subjected to 72 h of hypoxia became methotrexate resistant. In a similar study, amplification of the multiple-drug-resistance P-glycoprotein gene was also found to be responsible for the hypoxia-induced adriamycin resistance [21]. When hypoxia-treated cells were selected for resistance to both drugs, it was found that the frequency of simultaneously acquired double resistance was 10–100 times higher than if each resistance was selected independently. Thus, they proposed that the original event, i.e., DNA overreplication leading to the development of drug resistance, had to involve significant portions of the cellular genome. The subset of cells with overreplicated DNA were shown to not only acquire drug resistance, but also to have enhanced metastatic potential [29,32–34]. However, the mechanism underlying such gene amplification phenomenon is still controversial. For instance, Hahn et al. [35] proposed that chromosomal changes may occur as a result of asymmetric segregation of chromosome fragments or unequal sister chromatid exchange rather than DNA overreplication [36]. This alternative explanation could also account for the findings by Rofstad et al. [37] that hypoxia followed by reoxygenation may even induce tetraploidization of a diploid human melanoma cell line. Regardless of the mechanism, the tumor hypoxic state does seem to lead to increased cellular heterogeneity and malignant progression.

The aforementioned studies focused on large chromosomal rearrangements. To detect small-scale mutations that may be induced by hypoxia, Reynolds et al. [6] used a murine cell line that carried multiple copies of *λsupF*, a lambda phage shuttle vector containing the *supF* tRNA suppressor gene as the mutation reporter gene. This assay is particularly suitable for selecting small deletions, and it is well established that the *supF* gene can report all types of point mutations. It was found that following a 4-h exposure of the cells to severe hypoxia, the mutation frequency in the *supF* reporter gene was elevated 3.4-fold above that for the untreated cells grown parallel in normal aerated culture conditions. Multiple exposures to hypoxia with recovery between each treatment further enriched the mutation frequency, indicating a cumulative dose-response effect. These results suggest that under hypoxic conditions, damage to DNA, as well as to cellular replication and repair mechanisms, may occur, leading to mutations.

4. Nutrient deprivation and low pH

Much less attention has been devoted to the study of the other two important features of the tumor microenvironment, i.e., nutrient deprivation and acidity. Yet, both conditions can profoundly alter cell metabolism and physiology, and so potentially contribute to genetic instability in developing tumors.

Because metabolism is an energy-dependent process, cells deprived of glucose and other energy sources can have impaired activity of many enzymes, including DNA polymerases, topoisomerases, helicases, ligases, etc. Similarly, an acidic internal environment may also cause alterations in protein structure and function. This can lead to mutagenesis due to a decrease in fidelity of both DNA synthesis and repair. pH has been shown to have an effect on

several DNA polymerases [38,39]. Although these in vitro studies reported a slightly decreased error rate with respect to both base substitutions and frameshifts at low pH, this decreased error rate was attributed mainly to the drastically reduced processivity of the polymerase. In addition, the in vivo environment can be more complex; for example, it could be that the fluctuation of pH is more deleterious.

Both glucose-starvation and acidosis have been demonstrated to enhance metastatic potential of tumor cells [34,40], a possible reflection of genetic instability. Similar to the effect of hypoxia, when cells were exposed to pH 6.5 or starved for glucose followed by recovery in normal growth medium, a fraction of cells were found with overreplicated DNA [40]. Furthermore, exposure to acidosis also led to a small increase in methotrexate resistance in a murine tumor cell line [40].

A decreased perfusion rate deprives tumor cells not only of their energy source, but of other critical elements as well. Using a Chinese hamster ovary cell line carrying the *E. coli gpt* gene as the mutation reporter, Goncharova et al. [41] observed that serum deprivation induced a hypermutable state in those cells. Incubation of the cells in serum concentrations lower than 0.25% led to an almost 5-fold increase in the mutation rate. These cells had a higher level of intracellular oxidants compared to the level in control cells as demonstrated by DCF fluorescence measurements. When the low serum was supplemented with antioxidants, the mutation rate was reduced in a dose-dependent manner. These results suggest that the absence of antioxidants and other critical components due to the limited blood supply can create a hypermutagenic state within tumor cells.

5. Animal models

Studies using different culture conditions are important in dissecting out the components that are responsible for the genetic instability in developing tumors. However, such in vitro conditions do not exactly mimic the tumor's complex microenvironments, which vary spatially and temporally [6]. Moreover, different environmental factors may interact with one another synergistically, producing a bigger effect than each factor alone. Boyer and Tannock [42] found that hypoxia for 6 h had no effect on the regulation of intracellular pH, yet a decrease in extracellular pH as a result of chronic hypoxia did impair the activity of both the Na^+-dependent Cl^-/HCO_3^- and the Na^+/H^+ exchangers, and hence the recovery of the acidic internal environment. Rotin et al. [43] also reported that cellular ATP, energy charge and cell viability were reduced after a 6-h exposure to hypoxia at pH 6.0, but none were influenced by hypoxia or acid pH alone. These studies indicate that the *combined* effect of hypoxia and an acidic environment may be especially important in leading to changes in cellular energy metabolism, cell survival and possibly genomic integrity as well [15,43].

In this regard, the strong evidence suggesting a mutagenic effect of the complex tumor microenvironment comes from studies done directly with experimental tumors. Reynolds et al. [6] established an animal model in which the mouse cell line, LN12, carrying a chromosomally based λ phage shuttle vector was implanted subcutaneously into the flanks of nude mice to generate tumors. At the same time, equal numbers of LN12 cells were grown in vitro under standard culture conditions. A total of six tumors were collected, each displaying the spatial variation in oxygenation expected for solid tumors, with oxygen tensions varying from near those of venous blood to very severe hypoxia. The genomic DNA was prepared from these tumors, and incubated with λ in vitro packaging extracts to rescue the λ vector DNA for genetic analysis of the *supF* gene (Fig. 1). They found a total mutation frequency of 9.3×10^{-5} for the cells in the tumors vs. 1.8×10^{-5} for the cultured cells, a 5-fold elevation (Fig. 2). Since it has been shown in experimental tumor models that the tumor cell populations consistently have longer mean cell cycle times than do parallel samples grown in vitro under optimal culture conditions [44], calculation of mutation rates (if the actual number of cell divisions could be counted) would likely accentuate these differences. Thirty-one percent of the mutations in the tumors were deletions, whereas all the mutations in cultured cells were point mutations, implicating strand breakage as a major premutagenic lesion produced by the tumor microenvironment. The remaining 69% point mutations were characterized by a slight overrepresentation of

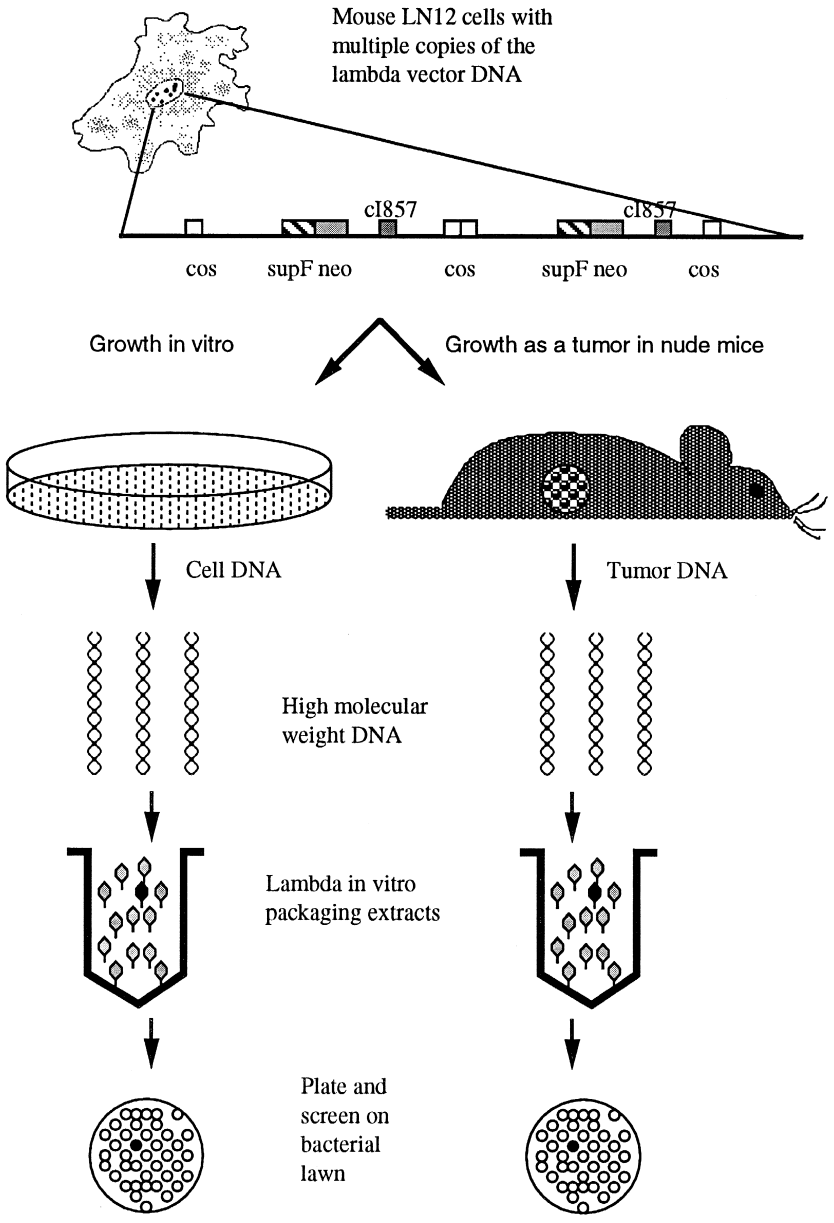

Fig. 1. Experimental protocol used by Reynolds et al. [6] to examine mutagenesis in tumor microenvironment. The tumorigenic mouse cells, LN12, carrying multiple copies of a chromosomally based λ phage shuttle vector, λ*supF*, were grown either in standard culture conditions or injected s.c. into flanks of mice to generate tumors. Genomic DNA was prepared and incubated with λ in vitro packaging extracts to rescue the λ vector DNA. Mutations in the *supF* gene were detected by growth of the rescued phage on an *E. coli* indicator strain.

transversions compared with those of controls, again indicating a qualitative abnormality in the maintenance of genomic integrity by cells growing within solid tumors. In another study [7], murine fibrosarcoma cells containing the *hprt* gene were grown as a subcutaneous tumor in nude mice. These tumor cells were later explanted and incubated in culture medium in the presence of 6-TG for the selection of *hprt*

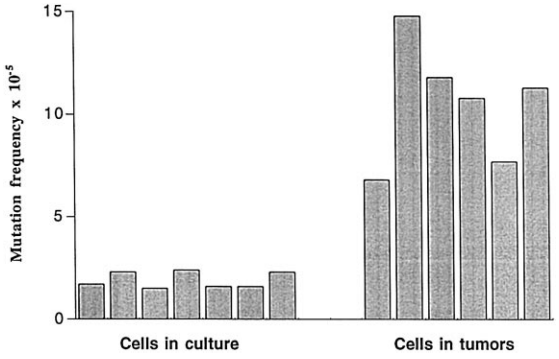

Fig. 2. Mutation frequencies of the *supF* gene detected in the λ*supF* vectors rescued from the LN12 cells grown as tumors vs. grown in culture. Each bar represents the frequency of mutations in phage vectors rescued from LN12 cells either prepared from an individual tumor or grown as a clonal population under standard culture condition. Data were adapted from Reynolds et al. [6].

mutants. The frequency of mutation in cells grown in one tumor was found to be 3.4-fold higher than in cells grown in tissue culture for an equivalent period of time.

6. Interaction of the tumor microenvironment and genetic factors

Growing evidence now points to a role of tumor microenvironments in the development of genetic instability. Tumor progression is a dynamic process. Factors intrinsic to tumor cells, such as loss of wild-type *p53* or mismatch repair genes, and those extrinsic to the cells are not mutually exclusive. Studies conducted by Graeber et al. [45] suggest that the tumor microenvironment and genetic factors may actually interact with each other during tumor evolution. They found that hypoxia led to substantial apoptosis in transformed rat fibroblasts. However, when these cells were genetically further altered either by overexpression of *bcl-2* or by knock out of *p53*, apoptotic response was significantly reduced. These results indicate that the genetic status of a tumor can modulate its susceptibility to environment-induced checkpoint mechanisms. On the other hand, hypoxia favors the survival of cells defective in apoptosis. These authors were able to show in cell mixing experiments that, after multiple rounds of hypoxic treatment, a small percentage of cells nullizygous for *p53* can outgrow similar cells expressing wild-type *p53*. Therefore, the tumor microenvironment can act as a selective agent favoring cells with a particular genetic composition, such as those that have lost apoptotic potential or acquired a growth advantage.

The conditional mutator phenotype reported by Richards et al. [5] is another example of how environmental cues may modulate mutation rate and genetic stability. In fact, the adverse environment experienced by the tumor cells may induce a transient expression of a mutator phenotype in the absence of an actual mutation in a repair gene. Recently, it was found that in *E. coli*, mismatch repair is downregulated during stationary-phase due to a functional deficiency of *mutL*, rather than to a decrease in the amount of the protein [46]. Similar work has not been done in a mammalian system. Nonetheless, it is reasonable to hypothesize that in developing tumors, when growth conditions are suboptimal, some critical proteins may be downregulated or modified to nonfunctional form, transiently permitting an increase in mutagenic events. Some of the cells may acquire advantageous mutations that allow them to overcome the restricted condition and to expand clonally [47]. This microenvironmental selection process for a suitable genetic alteration may therefore account for one aspect of tumor progression.

7. Discussion

The concept that ‘‘a mutator phenotype may be required for multistage carcinogenesis’’ proposed by Loeb is widely accepted [2]. The underlying mechanisms of the increased genomic instability during tumor progression have not been fully established. However, it is fair to propose that in addition to endogenous factors (such as disruption of genes involved in DNA repair), exogenous environmental factors to which a tumor is exposed may play an equally important role in promoting the evolution of a progressively malignant phenotype. The tumor microenvironment can contribute to mutations in several ways.

(1) It can cause DNA damage either by producing reactive oxygen radicals due to the hypoxia-reoxygenation injury, or by inducing endonucleases leading to strand breakage.

(2) The alteration in internal cellular environment, such as an increase in acidity and a decrease in energy stores, can have a profound effect on cellular metabolism and physiology. This may result in secondary modification of critical proteins that are involved in replication, repair or cell cycle regulation.

(3) The tumor microenvironment can further provide a selective pressure, encouraging the expansion of cell lineages carrying mutations that permit a clone to survive the adverse growth conditions. Some of the mutations which provide a growth advantage, such as *p53* disruption, may also further diminish the ability of the cell to maintain genomic integrity.

The importance of the tumor environment as a source of genetic instability in tumors has not received a large amount of attention in the past. In light of the emerging in vivo and in vitro studies reviewed here, a reexamination of tumor microenvironmental factors may aid us significantly in understanding the extent to which they contribute to phenomenon of tumor genetic instability and malignant progression.

Acknowledgements

We thank S. Rockwell, L. Guo, S. Peretz, P. Chan, L. Cabral, L. Narayanan, R. Franklin, and S.J. Baserga for their help. This work was supported by the American Cancer Society (VM189). J. Yuan is supported by an MD/PhD fellowship from the Yale University School of Medicine.

References

[1] P.C. Nowell, The clonal evolution of tumor cell populations, Science 194 (1976) 23–28.
[2] L.A. Loeb, Mutator phenotype may be required for multistage carcinogenesis, Cancer Res. 51 (1991) 3075–3079.
[3] C.E. Bronner et al., Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary nonpolyposis colon cancer, Nature 368 (1994) 258–261.
[4] C.C. Harris, M. Hollstein, Clinical implications of the p53 tumor-suppressor gene, New Engl. J. Med. 329 (1993) 1318–1327, [see comments].
[5] B. Richards, H. Zhang, G. Phear, M. Meuth, Conditional mutator phenotypes in hMSH2-deficient tumor cell lines, Science 277 (1997) 1523–1526.
[6] T.Y. Reynolds, S. Rockwell, P.M. Glazer, Genetic instability induced by the tumor microenvironment, Cancer Res. 56 (1996) 5754–5757.
[7] D. Wilkinson, J.K. Sandhu, J.W. Breneman, J.D. Tucker, H.C. Birnboim, *Hprt* mutants in a transplantable murine tumour arise more frequently in vivo than in vitro, Br. J. Cancer 72 (1995) 1234–1240.
[8] J.E. Moulder, S. Rockwell, Tumor hypoxia: its impact on cancer therapy, Cancer Metastasis Rev. 5 (1987) 313–341.
[9] S. Rockwell, Use of hypoxia-directed drugs in the therapy of solid tumors, Semin. Oncol. 19 (1992) 29–40.
[10] S. Rockwell, J.P.S. Knisely, Hypoxia and angiogenesis in experimental tumor models: Therapeutic implications 1, Birkhauser Verlag, Basel, 1996, in press.
[11] H. Kimura et al., Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma, Cancer Res. 56 (1996) 5522–5528.
[12] S. Rockwell, J.E. Moulder, Hypoxic fractions of human tumors xenografted into mice: a review, Int. J. Radiat. Oncol. Biol. Phys. 19 (1990) 197–202.
[13] F. Kallinowski et al., Glucose uptake, lactate release, ketone body turnover, metabolic micromilieu, and pH distributions in human breast cancer xenografts in nude rats, Cancer Res. 48 (1988) 7264–7272.
[14] J.L. Wike-Hooley, J. Haveman, H.S. Reinhold, The relevance of tumour pH to the treatment of malignant disease, Radiother. Oncol. 2 (1984) 343–366.
[15] I.F. Tannock, D. Rotin, Acid pH in tumors and its potential for therapeutic exploitation, Cancer Res. 49 (1989) 4373–4384.
[16] C.S. Heacock, R.M. Sutherland, Enhanced synthesis of stress proteins caused by hypoxia and relation to altered cell growth and metabolism, Br. J. Cancer 62 (1990) 217–225.
[17] T.G. Graeber et al., Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status, Mol. Cell. Biol. 14 (1994) 6264–6277.
[18] E.O. Pettersen, N.O. Juul, O.W. Ronning, Regulation of protein metabolism of human cells during and after acute hypoxia, Cancer Res. 46 (1986) 4346–4351.
[19] J.M. Brown, A.J. Giaccia, Tumour hypoxia: the picture has changed in the 1990s, Int. J. Radiat. Biol. 65 (1994) 95–102.
[20] O. Amellem, E.O. Pettersen, Cell inactivation and cell cycle inhibition as induced by extreme hypoxia: the possible role of cell cycle arrest as a protection against hypoxia-induced lethal damage, Cell Prolif. 24 (1991) 127–141.
[21] G.C. Rice, V. Ling, R.T. Schimke, Frequencies of independent and simultaneous selection of Chinese hamster cells for methotrexate and doxorubicin (adriamycin) resistance, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 9261–9264.
[22] H. Probst et al., Oxygen dependent regulation of DNA synthesis and growth of Ehrlich ascites tumor cells in vitro and in vivo, Cancer Res. 48 (1988) 2053–2060.

[23] C.R. Welbourn et al., Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil, Br. J. Surg. 78 (1991) 651–655.
[24] K.C. Cheng, D.S. Cahill, H. Kasai, S. Nishimura, L.A. Loeb, 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G–T and A–C substitutions, J. Biol. Chem. 267 (1992) 166–172.
[25] D.L. Stoler, G.R. Anderson, C.A. Russo, A.M. Spina, T.A. Beerman, Anoxia-inducible endonuclease activity as a potential basis of the genomic instability of cancer cells, Cancer Res. 52 (1992) 4372–4378.
[26] C.A. Russo et al., An anoxia inducible endonuclease and enhanced DNA breakage as contributors to genomic instability in cancer, Cancer Res. 55 (1995) 1122–1128.
[27] G.C. Rice, C. Hoy, R.T. Schimke, Transient hypoxia enhances the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 5978–5982.
[28] G.C. Rice, I.J. Spiro, C.C. Ling, Detection of S-phase overreplication following chronic hypoxia using a monoclonal anti-BrdUrd, Int. J. Radiat. Oncol. Biol. Phys. 11 (1985) 1817–1822.
[29] S.D. Young, R.S. Marshall, R.P. Hill, Hypoxia induces DNA overreplication and enhances metastatic potential of murine tumor cells, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 9533–9537.
[30] R.T. Schimke, S.W. Sherwood, A.B. Hill, R.N. Johnston, Overreplication and recombination of DNA in higher eukaryotes: potential consequences and biological implications, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 2157–2161.
[31] B.A. Teicher, Hypoxia and drug resistance, Cancer Metastasis Rev. 13 (1994) 139–168.
[32] M. Hockel et al., Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix, Cancer Res. 56 (1996) 4509–4515.
[33] D.M. Brizel et al., Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma, Cancer Res. 56 (1996) 941–943.
[34] C. Cuvier, A. Jang, R.P. Hill, Exposure to hypoxia, glucose starvation and acidosis: effect on invasive capacity of murine tumor cells and correlation with cathepsin (L + B) secretion, Clin. Exp. Metastasis 15 (1997) 19–25.
[35] P. Hahn, L.N. Kapp, W.F. Morgan, R.B. Painter, Chromosomal changes without DNA overproduction in hydroxyurea-treated mammalian cells: implications for gene amplification, Cancer Res. 46 (1986) 4607–4612.
[36] R.B. Painter, B.R. Young, L.N. Kapp, Absence of DNA overreplication in Chinese hamster cells incubated with inhibitors of DNA synthesis, Cancer Res. 47 (1987) 5595–5599.
[37] E.K. Rofstad, N.M. Johnsen, H. Lyng, Hypoxia-induced tetraploidisation of a diploid human melanoma cell line in vitro, Br. J. Cancer 74 (27) (1996) S136–S139.
[38] K.A. Eckert, T.A. Kunkel, Fidelity of DNA synthesis catalyzed by human DNA polymerase alpha and HIV-1 reverse transcriptase: effect of reaction pH, Nucleic Acids Res. 21 (1993) 5212–5220.
[39] K.A. Eckert, T.A. Kunkel, Effect of reaction pH on the fidelity and processivity of exonuclease-deficient Klenow polymerase, J. Biol. Chem. 268 (1993) 13462–13471.
[40] O.K. Schlappack, A. Zimmermann, R.P. Hill, Glucose starvation and acidosis: effect on experimental metastatic potential, DNA content and MTX resistance of murine tumour cells, Br. J. Cancer 64 (1991) 663–670.
[41] E.I. Goncharova, A. Nadas, T.G. Rossman, Serum deprivation, but not inhibition of growth per se, induces a hypermutable state in Chinese hamster G12 cells, Cancer. Res. 56 (1996) 752–756.
[42] M.J. Boyer, I.F. Tannock, Regulation of intracellular pH in tumor cell lines: influence of microenvironmental conditions, Cancer Res. 52 (1992) 4441–4447.
[43] D. Rotin, B. Robinson, I.F. Tannock, Influence of hypoxia and an acidic environment on the metabolism and viability of cultured cells: potential implications for cell death in tumors, Cancer Res. 46 (1986) 2821–2826.
[44] S. Rockwell, In vivo-in vitro tumor systems: New models for studying the response of tumors to therapy, Lab. Anim. Sci. 27 (1977) 831–851.
[45] T.G. Graeber et al., Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours, Nature 379 (1996) 88–91.
[46] R.S. Harris et al., Mismatch repair protein MutL becomes limiting during stationary-phase mutation, Genes Dev. 11 (1997) 2426–2437.
[47] L.A. Loeb, Transient expression of a mutator phenotype in cancer cells, Science 277 (1997) 1449–1450, [comment].