Biochemical and Biophysical Research Communications 396 (2010) 147–151

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Horizontal gene transfer: You are what you eat

Lars Holmgren

Department of Oncology and Pathology, Karolinska Institutet Stockholm, R8:03 CCK, Karolinska Hospital, SE 17176 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 22 March 2010

Keywords:
Apoptosis
Phagocytosis
Gene transfer
Neoplasia

ABSTRACT

Horizontal or lateral gene transfer is an effective mechanism for the exchange of genetic information in bacteria allowing bacterial diversification and facilitating adaptation to new environments. Recent data demonstrate that DNA may also be transferred between somatic cells via the uptake of apoptotic bodies. This process allows transfer of viral genes that have been incorporated into the genome in a receptor-independent fashion. Transferred DNA is replicated and propagated in daughter cells in cell that have an inactivated DNA response which may impact tumor progression.

1. Introduction

The concept of cellular apoptosis was initially described by Kerr and Wyllie and has ever since drawn increasing attention [1]. Much of the interest stems for the pivotal role of apoptosis during embryonic development and the control of organ homeostasis [2,3]. Local competition for growth factors leads to the death of cells that have not responded to survival cues, a process that is often associated organs and tissue morphogenesis, e.g., atrophy of interdigital tissue during limb development. In adult tissues, apoptosis is one of the mediatory processes for maintaining homeostasis. A typical example is the small intestine where 10^9 apoptotic cells are sloughed off every hour into the lumen and removed by the GI tract [4]. Disturbance in the delicate balance together with alterations in the microenvironment can lead to uncontrolled cell proliferation and potential carcinogenesis [5].

Apoptosis *in vivo* is quite inconspicuous due to the rapid elimination of the cellular remnants [6]. This is exemplified within the thymus where over 90% of thymocytes die during thymic selection but only a minute fraction can be detected using techniques that visualize DNA fragmentation [7]. Dying cells are removed by two primary pathways. Firstly, they are excreted through shedding into a different compartment which seems to a general trait of the epithelium as this can be observed in keratinocytes of the skin, lung epithelium and the kidney. Alternately, moribund cells in the midst of living cells are cleared through engulfment by macrophages or other phagocytes. During early apoptotic process phosphatidylserine is externalized thus marking the cell to be endocytosed. The elimination of apoptotic bodies involves a number of molecular pathways that have been reviewed elsewhere [8,9]. Once inside the engulfing cell the apoptotic body is transferred into lyzosomes and degraded. Taken together, apoptosis is a means of specifically and unobtrusively eliminating unwanted cells. This has been the current view but research by my colleagues and I as well as other investigators have challenged this dogma.

2. The backdrop for horizontal gene transfer

Is it possible that DNA from dying cells can be recovered and recycled? In the mid-nineties Judah Folkman co-workers discovered that mouse lung metastasis could be maintained in a state of dormancy if angiogenesis was suppressed [10]. These metastases circumscribed pre-existing lung venules and did not grow beyond a diameter of 250 μm. Further analysis showed that these tumor cells in fact were not in a state of latency as they were actively proliferating. The reason for the apparent stasis was that within these micro-tumors generation of new cells was balanced by cell death through apoptosis. Once inhibition of angiogenesis was reversed, apoptosis levels reduced and the metastasis grew and killed the host within weeks. The results indicated that massive amounts of apoptotic cells could be cleared within the micro-metastasis with an impressive turnover of DNA. Since in view of the inherent genomic instability and heterogeneity of most cancers, could uptake of DNA from dying tumors cells be a driving force? Transfer of genes could be a means of rapidly acquiring new traits such as chemotherapy resistance.

3. Horizontal gene transfer of viral genes

Where lies the evidence for this paradigm? Clearly there were challenges associated with establishing model systems which included distinguishing between donor and host cells and more specifically DNA from dying cells with that of the phagocyte. We decided to use lymphocytes with chromosomally integrated

E-mail address: lars.holmgren@ki.se

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.04.026

non-virus producing copies of the Epstein Barr virus (EBV) as donor cells [11]. By inducing apoptosis and adding the dying donor cells to human macrophages, endothelial cells and fibroblasts it was possible to detect expression of EBV-encoded genes in the new host. Frequency of expression correlated distinctly with phagocytotic activity wherein over 50% of macrophages and less than 0.01% of smooth muscle cells showed detectable expression. The observations however did not formally exclude mRNA or protein transfer. The next challenge was to find a method to distinguish between the two populations of DNA. For this purpose we used species-specific chromosome painting probes. When utilized together with EBV DNA probes we could validate the data with regard to transfer of genomic and viral DNA. There was a high frequency of transfer of human DNA into recipient bovine endothelial cells. Over 15–20% of the cells contained both human and EBV DNA indicating that this was a surprisingly efficient mode of DNA transfer. Taken together these experiments initiated the possibility of gene transfer via the uptake of apoptotic bodies.

With a similar strategy it was possible to transfer integrated copies of the HIV virus to human fibroblasts and immature dendritic cells [12]. The transfer of DNA to antigen-presenting cells opens up the possibility of prolonged expression and antigen presentation. Indeed, apoptotic bodies have been successfully used to vaccinate against HIV in a mouse model system and may be strategy to vaccinate against HIV in humans [12–14] (http://www.avaris.se/).

These findings may potentially explain why viruses such as EBV and HIV have been detected in cells that normally are not infectable by the virus. It also raises concerns with regard to xenotransplantation, i.e., transplantation between species. As there is a shortage of human donor organs, animal materials are being progressively investigated as an alternative source for organs. There is a potential risk of transmittance of viral genes that are integrated into the genome of the donor animal which then could be transferred to human cells if death is induced by apoptosis and then phagocytosed. Evidence for porcine endogenous retrovirus (PERV) infection of human cells has provoked a public health debate over the proposed use of porcine xenografts to alleviate the worldwide shortage of human allografts. Bisset et al. addressed this by co-culturing human cells with porcine donor cells and could find that 0.22% of the cells contained porcine DNA [15]. This may seem low but in view that the recipient cells were low phagocytotic fibroblasts and that macrophages and endothelial cells have a more than ten times higher efficiency in taking up DNA the findings needs serious deliberation.

Cell transplantation has also been tested to improve the regeneration of the myocardium after infarction. Injections of cells that improve the regeneration of the myocardium have been outlined as a promising approach. Interestingly, earlier reports suggested that injection of human endothelial cells have the potential to trans differentiate into myocytes. However, Burghoff and coworkers showed that following the death of transplanted, EGFP-expressing endothelial cells the cardiomyocytes picked up the EGFP label [16]. Their data suggest that DNA was transferred from the dying endothelial cells to the cardiomyocytes. The effect of this manner of DNA transfer for cardiac activity remains presently undetermined.

4. Horizontal gene transfer and tumor progression

The studies of viral gene transfer opened up the possibility that apoptotic bodies may be a vehicle for DNA transfer. However, we could not find evidence for chromosomal integration or replication during cell division in any of our experiments. Propagation of the acquired DNA and inheritance into daughter cells would be expected to occur in the event that DNA transfer has any role in oncogenesis. Could there be mechanism that protects normal cells from propagating exogenous DNA which presumably is lost on cell transformation? To address this question we followed the fate of BrdU-labeled DNA in apoptotic bodies after phagocytosis. As shown in Fig. 1 the apoptotic cells were observed juxtaposed and impinging upon the nuclear membrane of the recipient cell. Subsequently, the BrdU-positive DNA was detected within the nuclear cage. The transfer of DNA into the nucleus triggers activation of MRE11 and gamma histone 2HX which bind double strand breaks (Fig. 2, Ehnfors et al., unpublished data). Furthermore, engulfment of apoptic cells also activated p53 as analyzed by immunofluorescence and western blot. Since the p53 is mutated or absent in over 50% of all human tumors it opens up the possibility that tumors unlike normal cells could propagate apoptotic DNA.

We tested whether a functional p53 gene protects cells from propagating foreign DNA, whether these cells can be transformed and whether DNA can confer resistance to cytotoxic drugs. Ras and myc transformed rat fibroblasts were used as donor and primary wild type or p53-deficient mouse fibroblasts as recipient cells [17]. The conclusions from these experiments were quite clear. Loss of p53 allowed transfer of the ras and myc oncogenes resulting in foci formation *in vitro*. Even though the ras and myc oncogenes were transferred to the new host the DNA was lost over time in culture. This was also the case for drug resistance genes. Drug resistance genes were lost without selection pressure but could be maintained indefinitely under selective pressure. Thus, by adding *in vivo* selection pressure not only did the initially non-tumorigenic $p53^{-/-}$ cells form tumors, but the transferred oncogenes were also carried forward during tumor growth.

5. The role of DNase II and p53 activation

As discussed above, one of the apparent tasks of p53 among many is preventing cells from replicating DNA transferred from dying cells. How is this protective function regulated? As DNA fragmentation is a hallmark of apoptosis it is seems likely that fragmented DNA could be detected as DNA damage. It has been shown that two major systems are responsible for the cleavage of DNA during apoptosis. CAD (Caspase-Activated DNase) is normally associated with its inhibitor ICAD and localized both localized in the cytoplasm [18]. Upon caspase activation, ICAD is cleaved and dissociates from CAD which then enters the nucleus where it excises DNA. If CAD activity is inhibited, DNA degradation still occurs due to the presence of DNase II present in the lysosome of the phagocytosing cells. We inhibited autonomous DNA degradation within cells driven to apoptosis by transfecting them with a caspase-resistant ICAD construct. This efficiently inhibited DNA fragmentation but only delayed p53 activation in the recipient cells. The combination of blocking both CAD and DNaseII activity (using knock-out mouse embryonic fibroblasts) completely inhibited p53 activation which allowed propagation of transferred DNA with a similar frequency to that of p53-deficient cells [19]. The observations suggest that these two enzymes work together with p53 to form a genetic barrier blocking the replication of potentially harmful DNA introduced by apoptotic bodies (Fig. 3).

6. Aneuploidy of tumor endothelial cells

Our *in vitro* data introduced the possibility of gene transfer to tumors *in vivo*. To discriminate between donor and recipient cells more easily, we decided to study the transfer of genes between the tumor and endothelial cells of the tumor stroma. The control of blood vessel formation is clearly of importance as angiogenesis is a requirement for a tumor achieve clinical significance. During

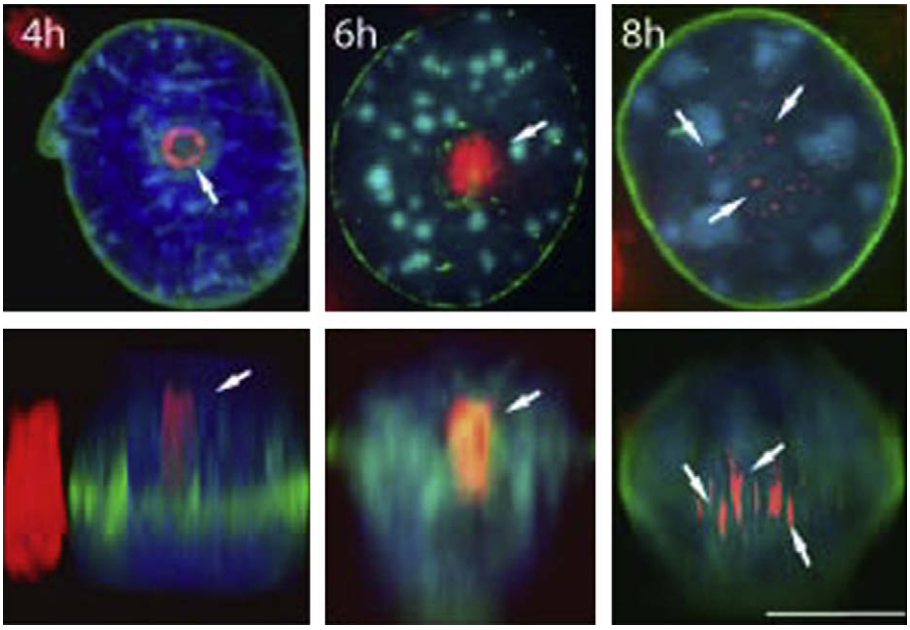

Fig. 1. Transfer of apoptotic DNA into the nucleus of phagocytosing mouse fibroblasts. DNA in rat embryonic fibroblast (REFrm) cells was labeled with the thymine analog BrdU before apoptosis was induced by nutrient depletion. The apoptotic cells were then added to mouse embryonic fibroblast (MEF) cells grown on chamber slides. At indicated time points the slides were fixed and stained for BrdU (red) and LaminB (green), which spans the nuclear inner membrane. DNA is labeled with DAPI (blue). Four to six hours after the apoptotic cells were added to MEF cells, DNA positive for BrdU was observed pressing and deforming the nuclear membrane of the phagocytosing MEF cells. Eight hours after co-cultivation of apoptotic REFrm cells and MEF cells, BrdU positive staining was seen in the nucleus of the phagocytosing MEF cells. Images were sampled in the *z*-axis and subsequently processed with 3D rendering software (velocity) to generate three-dimensional images. The images were viewed at a 90° angle to verify the location of the BrdU staining. Size bar = 10 μm.

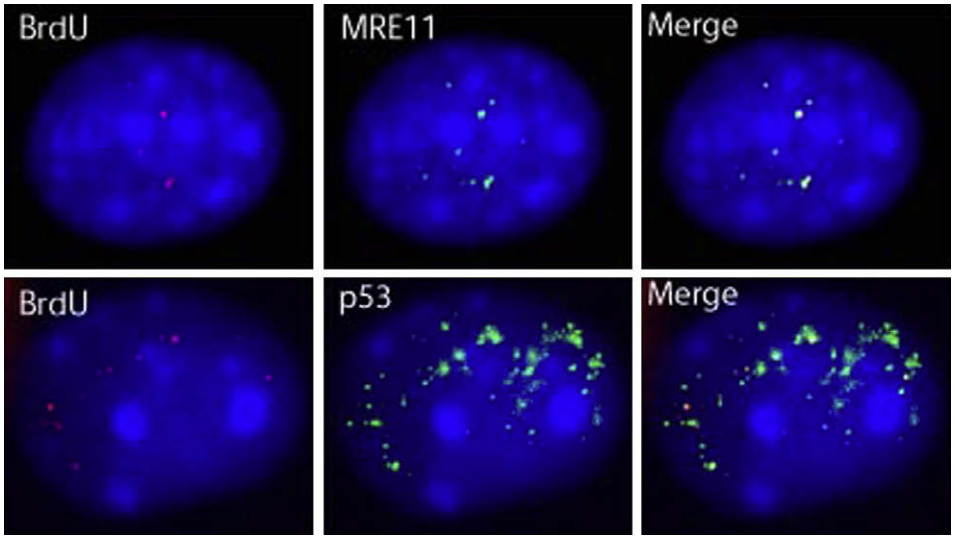

Fig. 2. DNA from apoptotic cells co-localize with early markers of DNA double strand breaks in the recipient host nucleus. The donor DNA was labeled with BrdU as shown in red (white arrow) and double stained with either antibodies specific for MRE11(which associates to damaged DNA) top panel or p53 bottom panel. CDNA is labeled with DAPI (blue). Images were sampled in the *z*-axis and subsequently processed with a 3D rendering software (velocity). Size bar equals 10 μm.

the last twenty years endothelial cells have become the targets for anti-angiogenic therapy [20,21]. Tumor resistance is a major problem in treating cancer patients with conventional therapies. Endothelial cells have been considered genetically stable and therefore potentially less prone to mutation and acquisition of resistance. Indeed, several anti-angiogenic therapies have received regulatory approval for treatment of solid tumors such as colorectal, breast, lung and renal cancers. One of the prototypes is Avastin (bevacizumab) which is a monoclonal antibody targeting vascular endothelial growth factor (VEGF) [22]. However, treatment of metastatic disease has not revealed any long-term tumor suppressive effects. Rather, the tumors appear to be come refractory to treatment. There may be several reasons for this; targeting one individual signaling pathway may select for tumor cells that can activate blood vessel formation via alternative pathways [23,24] or tumor endothelial cells may be inherently different than their counterparts in normal tissues [25]. This speculation is supported by recent observations that endothelial cells isolated from liver cancer patients display increased resistance to the chemotherapeutic agents 5-Fu and Adriamycin [26].

Recent findings have also challenged the notion that the tumor endothelial cells are genomically stable as endothelial from exper-

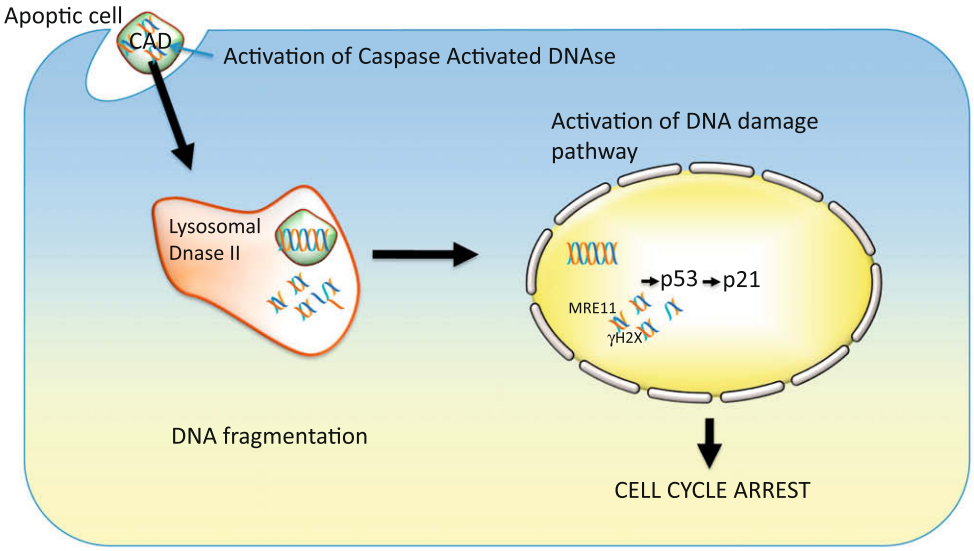

Fig. 3. Schematic figure showing the components activa in triggering the p53 response in phagocytosing cells. CAD is activated during apoptosis in the dying cells after phagocytosis, DNase II residing in the lysosome of the recipient continues to fragment incoming DNA. These DNA fragments activate the DNA damage response in the host thus preventing potential integration and propagation of foreign DNA. This models thus suggests that the Chk2/p53/p21 DNA damage pathway in the phagocytosing cell, together with the DNase II enzyme, form a genetic barrier.

imental tumors displayed aneuploidy [27]. In concordance, Streubel et al. found that micro-vascular endothelial cells in B-cell lymphomas harbored lymphoma-specific, genetic aberrations [28]. Taken together, these reports open up the possibility of plasticity between the tumor and stromal compartments.

7. Transfer of tumor DNA to endothelial cells

The replication of tumor DNA would require that p53 is inactivated either by a dominant-negative effect or by loss of p53 in the stroma. The latter possibility is supported by a number of studies showing that the tumor stroma harbors genetic mutations and altered DNA copy numbers [29]. Furthermore, p53 mutations have been detected in adjoining non-malignant tissues in diverse malignancies. Patoc et al. showed that specific loss of heterozygosity was associated with somatic p53 mutations and regional lymph node metastases in sporadic breast cancer but not in hereditary breast cancer [30]. These studies should be interpreted with caution as tumor contamination of the isolated tumor stroma was not unequivocally excluded [31].

To inhibit p53 response in recipient cells, we generated tumor cells expressing SV40LT (which inhibits p53 and Rb tumor suppressor function). Using these cells as donors it was now possible to transfer DNA via uptake of apoptotic bodies to normal, p53-positive, fibroblasts and endothelial cells *in vitro*. The transferred DNA was successfully propagated as evaluated by FISH analysis. Endothelial cells maintained the expression of endothelial markers but also expressed the SV40LT gene (Fig. 4). FISH analysis showed that the endothelial cells contained not only the SV40LT gene but also whole chromosomes from the tumor donor. The chromosomes were frequently fused to chromosomes of the endothelial host. These data showed that it was possible to transfer a p53 dominant negative gene that could suppress the recipient cells response to the transferred DNA [32]. We then subcutaneously injected SV40LT-positive and negative tumors in mice to assess whether tumor DNA could also occur *in vivo*. Tumors were resected, sectioned and subjected to FISH analysis. Approximately 4–5% of the stromal cells contained the SV40LT gene and were positive for the mouse-specific probe [32]. To eliminate artifacts ensuing from DNA smearing during cryo-sectioning, we analyzed tumor and stromal

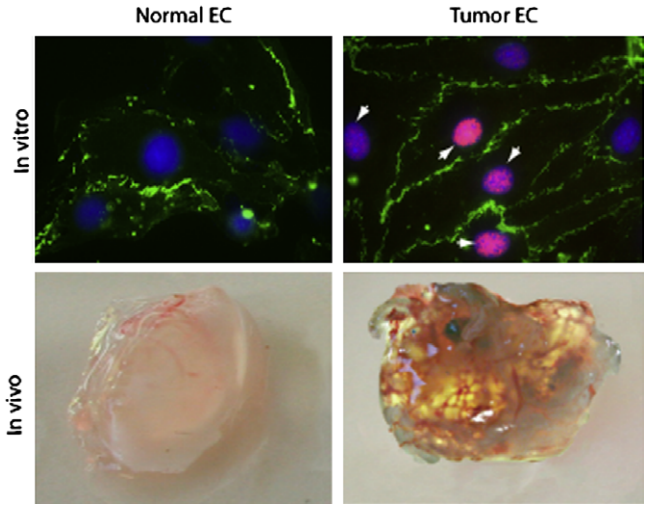

Fig. 4. Tumor-derived endothelial cells express tumor markers and maintain functionality *in vivo*. Magnetic beads coated with CD31/PECAM1 were used to isolate tumor endothelial cells (T-EC) from tumors established from rat fibrosarcomas expressing SV40 large T. Twenty isolated clones were positive for the endothelial surface markers CD31/PECAM1, CD34, VE-cadherin and VEGFR2, but negative for the immature hematopoietic stem cell marker CD133 and rat-MHC I. top panel shows data from one representative clone that isdouble-positive for PECAM1 (green) and SV40LT (red). To assess whether the isolated tumor-associated endothelial cells (T-ECs) were functional, the endothelial cells were mixed with Matrigel and injected into severe combined immunodeficient (SCID) mice. The resulting Matrigel plug showed blood-filled spaces and large blood vessels (bottom right image). In contrast, addition of primary mouse lung endothelial cells (L-ECs) did not promote angiogenesis when compared with control plugs.

cells isolated by collagenase digestion. FISH analysis established that 5.6% of the cells that hybridized the murine-specific probe were also positive for SV40 LT. This indicates that horizontal gene transfer between tumor and host stroma does indeed occur.

Over 20 tumor-derived endothelial cell lines were isolated. Each of these cell lines continued to express endothelial markers such as PECAM, VE-Cadherin and CD34 for over 30 passages *in vitro* concurrently with SV40LT expression. An important aspect was the functional ability of the endothelial cells containing tumor DNA

to actively participate in the formation of a microcirculatory network. Tube-forming capacity of T-EC *in vivo* was examined using an assay in which Matrigel plugs containing T-EC were implanted subcutaneously into SCID mice. T-EC embedded with Matrigel formed small blood-containing cysts. The inclusion of FGF-2 resulted in massive vascularization with large blood-filled vessels visible to the naked eye. Addition of primary mouse lung endothelial cells (L-ECs) did not promote angiogenesis compared to control plugs. These data show that tumor endothelial cells that contain tumor DNA are capable of forming a vessel network. The results are remarkable as they may represent a novel mechanism by which tumors affect blood vessel formation.

8. Concluding remarks

The data reviewed in this article suggest that apoptotic cells may be a vehicle for transfer of DNA. Uptake of partially fragmented foreign DNA triggers a DNA damage response and thus prevents replication of potentially harmful DNA. The fragmentation of the DNA is induced by the combined actions of CAD and DNase II enzymes. Interestingly, CAD has been shown to act as a tumor suppressor as CAD deficient mice have an increased risk to develop cancer and CAD is frequently mutated in human cancer [33–35]. It is not known whether DNase II deficiency results in increased susceptibility to cancer as DNase II knock-out mice die in utero [36]. It would be of interest to examine whether conditional inactivation of DNase II would confer increased genomic instability during tumor progression. The data generates the possibility that gene transfer promotes genetic instability in tumors or its adjacent stroma. The presence or absence of p53 in the stroma is still equivocal. However, if these data are validated p53 measurement in the tumor stroma may be an indirect indicator whether the tumor may actually be able to genetically manipulate its microenvironment.

Acknowledgments

L.H. is supported by grants from the Swedish Cancer Society, the Cancer Society of Stockholm, the Swedish Research Council, and EUCAAD 200755 of the Seventh Framework programme of the European Commission.

References

[1] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[2] D.L. Vaux, S.J. Korsmeyer, Cell death in development, Cell 96 (1999) 245–254.
[3] M.D. Jacobson, M. Weil, M.C. Raff, Programmed cell death in animal development, Cell 88 (1997) 347–354.
[4] C.S. Potten, The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice, Cancer Metastasis Rev. 11 (1992) 179–195.
[5] A.H. Wyllie, Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview, Cancer Metastasis Rev. 11 (1992) 95–103.
[6] W. Bursch, S. Paffe, B. Putz, G. Barthel, R. Schulte-Hermann, Determination of the length of the histological stages of apoptosis in normal liver and in altered hepatic foci of rats, Carcinogenesis 11 (1990) 847–853.
[7] C.D. Surh, J. Sprent, T-cell apoptosis detected in situ during positive and negative selection in the thymus, Nature 372 (1994) 100–103.
[8] B. Fadeel, Programmed cell clearance, Cell. Mol. Life Sci. 60 (2003) 2575–2585.
[9] J. Savill, V. Fadok, Corpse clearance defines the meaning of cell death, Nature 407 (2000) 784–788.
[10] L. Holmgren, M.S. O'Reilly, J. Folkman, Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression, Nat. Med. 1 (1995) 149–153.
[11] L. Holmgren, A. Szeles, E. Rajnavolgyi, J. Folkman, G. Klein, I. Ernberg, K.I. Falk, Horizontal transfer of DNA by the uptake of apoptotic bodies, Blood 93 (1999) 3956–3963.
[12] A.L. Spetz, B.K. Patterson, K. Lore, J. Andersson, L. Holmgren, Functional gene transfer of HIV DNA by an HIV receptor-independent mechanism, J. Immunol. 163 (1999) 736–742.
[13] A.L. Spetz, A.S. Sorensen, L. Walther-Jallow, B. Wahren, J. Andersson, L. Holmgren, J. Hinkula, Induction of HIV-1-specific immunity after vaccination with apoptotic HIV-1/murine leukemia virus-infected cells, J. Immunol. 169 (2002) 5771–5779.
[14] A.L. Spetz, J. Strominger, V. Groh-Spies, T cell subsets in normal human epidermis, Am. J. Pathol. 149 (1996) 665–674.
[15] L.R. Bisset, J. Boni, H. Lutz, J. Schupbach, Lack of evidence for PERV expression after apoptosis-mediated horizontal gene transfer between porcine and human cells, Xenotransplantation 14 (2007) 13–24.
[16] S. Burghoff, Z. Ding, S. Godecke, A. Assmann, A. Wirrwar, D. Buchholz, O. Sergeeva, C. Leurs, H. Hanenberg, H.W. Muller, W. Bloch, J. Schrader, Horizontal gene transfer from human endothelial cells to rat cardiomyocytes after intracoronary transplantation, Cardiovasc. Res. 77 (2008) 534–543.
[17] A. Bergsmedh, A. Szeles, M. Henriksson, A. Bratt, M.J. Folkman, A.L. Spetz, L. Holmgren, Horizontal transfer of oncogenes by uptake of apoptotic bodies, Proc. Natl. Acad. Sci. USA 98 (2001) 6407–6411.
[18] S. Nagata, H. Nagase, K. Kawane, N. Mukae, H. Fukuyama, Degradation of chromosomal DNA during apoptosis, Cell Death Differ. 10 (2003) 108–116.
[19] A. Bergsmedh, J. Ehnfors, K. Kawane, N. Motoyama, S. Nagata, L. Holmgren, DNase II and the Chk2 DNA damage pathway form a genetic barrier blocking replication of horizontally transferred DNA, Mol. Cancer Res. 4 (2006) 187–195.
[20] J. Folkman, Tumor angiogenesis: therapeutic implications, N. Engl. J. Med. 285 (1971) 1182–1186.
[21] J. Folkman, Angiogenesis in cancer, vascular, rheumatoid and other disease, Nat. Med. 1 (1995) 27–31.
[22] N. Ferrara, K.J. Hillan, W. Novotny, Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy, Biochem. Biophys. Res. Commun. 333 (2005) 328–335.
[23] M. Relf, S. LeJeune, P.A. Scott, S. Fox, K. Smith, R. Leek, A. Moghaddam, R. Whitehouse, R. Bicknell, A.L. Harris, Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis, Cancer Res. 57 (1997) 963–969.
[24] O. Casanovas, D.J. Hicklin, G. Bergers, D. Hanahan, Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors, Cancer Cell 8 (2005) 299–309.
[25] B. St Croix, C. Rago, V. Velculescu, G. Traverso, K.E. Romans, E. Montgomery, A. Lal, G.J. Riggins, C. Lengauer, B. Vogelstein, K.W. Kinzler, Genes expressed in human tumor endothelium, Science 289 (2000) 1197–1202.
[26] Y.Q. Xiong, H.C. Sun, W. Zhang, X.D. Zhu, P.Y. Zhuang, J.B. Zhang, L. Wang, W.Z. Wu, L.X. Qin, Z.Y. Tang, Human hepatocellular carcinoma tumor-derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells, Clin. Cancer Res. 15 (2009) 4838–4846.
[27] K. Hida, M. Klagsbrun, A new perspective on tumor endothelial cells: unexpected chromosome and centrosome abnormalities, Cancer Res. 65 (2005) 2507–2510.
[28] B. Streubel, A. Chott, D. Huber, M. Exner, U. Jager, O. Wagner, I. Schwarzinger, Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas, N. Engl. J. Med. 351 (2004) 250–259.
[29] J. Bar, N. Moskovits, M. Oren, Involvement of stromal p53 in tumor-stroma interactions, Semin. Cell Dev. Biol. (2009).
[30] A. Patocs, L. Zhang, Y. Xu, F. Weber, T. Caldes, G.L. Mutter, P. Platzer, C. Eng, Breast-cancer stromal cells with TP53 mutations and nodal metastases, N. Engl. J. Med. 357 (2007) 2543–2551.
[31] C. Holliday, S. Rummel, J.A. Hooke, C.D. Shriver, D.L. Ellsworth, R.E. Ellsworth, Genomic instability in the breast microenvironment? A critical evaluation of the evidence, Expert Rev. Mol. Diagn. 9 (2009) 667–678.
[32] J. Ehnfors, M. Kost-Alimova, N.L. Persson, A. Bergsmedh, J. Castro, T. Levchenko-Tegnebratt, L. Yang, T. Panaretakis, L. Holmgren, Horizontal transfer of tumor DNA to endothelial cells in vivo, Cell Death Differ. 16 (2009) 749–757.
[33] B. Yan, H. Wang, Y. Peng, Y. Hu, X. Zhang, Q. Chen, J.S. Bedford, M.W. Dewhirst, C.Y. Li, A unique role of the DNA fragmentation factor in maintaining genomic stability, Proc. Natl. Acad. Sci. USA 103 (2006) 1504–1509.
[34] B. Yan, H. Wang, D. Zhuo, F. Li, T. Kon, M. Dewhirst, C.Y. Li, Apoptotic DNA fragmentation factor maintains chromosome stability in a P53-independent manner, Oncogene 25 (2006) 5370–5376.
[35] P. Widlak, W.T. Garrard, Roles of the major apoptotic nuclease-DNA fragmentation factor-in biology and disease, Cell. Mol. Life Sci. 66 (2009) 263–274.
[36] K. Kawane, H. Fukuyama, G. Kondoh, J. Takeda, Y. Ohsawa, Y. Uchiyama, S. Nagata, Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver, Science 292 (2001) 1546–1549.