[Frontiers in Bioscience 14, 2704-2713, January 1, 2009]

Horizontal DNA and mRNA transfer between donor and recipient cells after allogeneic hematopoietic cell transplantation?

Miguel Waterhouse[1], Maria Themeli[2], Yannis Metaxas[1], Eleni D Lagadinou[2], Jurgen Finke[1], Alexandros Spyridonidis[1, 2]

[1]Department of Hematology and Oncology, Albert-Ludwigs University Medical Center, Freiburg, Germany, [2]Division of Hematology, University of Patras Medical Center, Patras, Greece

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Epithelial chimerism after allogeneic HCT
4. Genomic instability in epithelium after allogeneic HCT
5. Horizontal gene transfer
6. Origin and composition of extracellular genetic material
7. Nucleic acid uptake by the cells
8. Fate of the foreign genetic material within the cell
9. Summary and perspective
10. Acknowledgements
11. References

1. ABSTRACT

Allogeneic hematopoietic cell transplantation in humans results in true biological chimeras. There is now accumulating evidence that besides Graft versus Host Disease (GvHD), there are also other consequences in the co-existence of two genetically distinct populations in the transplant recipient. First, epithelial cells with donor-derived genotype emerge. Second, epithelial tissues of the host acquire genomic alterations. The current review discusses existing data on these recently discovered phenomena and focuses on horizontal gene transfer between donor and recipient cells as a possible mechanism explaining and linking these phenomena.

2. INTRODUCTION

Bone marrow (BM) transplantation has become a standard treatment for many hematological malignancies (1). Continuous experimental and clinical research led to characterization and identification of the hematopoietic stem cells (HSC) within the BM leading to the refinement of this procedure which is now called Hematopoietic Stem Cell Transplantation (HSCT) or Hematopoietic Cell Transplantation (HCT) (2). During allogeneic HCT (allo-HCT) the recipient receives a preparative conditioning regimen (e.g. chemotherapy, radiotherapy) to destroy his hematopoiesis and immune system. This practice is followed by the administration of HSC harvested from the

donor. The donor's HSC engraft, proliferate and finally reconstitute hematopoiesis in the recipient (3, 4). The result is the creation of a biological chimera, a term used to describe the presence of tissues of different genetic origin in the same organism; the hematopoietic cells derive from the donor whilst the other tissues (e.g. epithelium) are genetically derived from the patient-recipient. This un-physiological formation of biological chimeras is not free of consequences. The first sequel which has been recognized in the development of chimeric organisms after allo-HCT is the graft versus host disease (GvHD) in which the new developed immune cells from the graft recognize the host's epithelial cells as foreign and kill them (5). There is now accumulating evidence that there are also other consequences of the co-existence of two genetically distinct populations in the transplant recipient. First, epithelial cells with donor-derived genotype emerge, a phenomenon which was initially misinterpreted and falsely described as "stem cell plasticity" (reviewed in (2)). Second, epithelial tissues of the host acquire genomic alterations (6). In the following we first briefly review these recently discovered phenomena occurring after allogeneic HCT and then discuss horizontal gene transfer (HGT) between donor and recipient cells as a possible mechanism explaining and linking these phenomena.

3. EPITHELIAL CHIMERISM AFTER ALLOGENEIC HCT

Although bone marrow transplantation (BMT) has been a part of clinical practice for more than 30 years, it was only recently realized that hematopoietic cellular infusions used in clinical practice may generate "unexpected" epithelial cell populations *in vivo* (7, 8). The revolutionary report of Ferrari in 1998 (9) suggesting that BM may be the source of muscle-specific stem cells was followed by a series of papers suggesting that when unfractionated BM cells or BM cell subsets with high hematopoietic activity were infused into irradiated mice, some of them were found again as different epithelial and mesenchymal cells like hepatocytes, skin cells, pneumocytes, intestinal epithelium, kidney epithelium, pancreas cells, skeletal muscle, myocardium, neurons, and endothelium (10, 2, 11, 12). These experimental animal results led to the exploration of similar events in humans and many studies suggested the generation of donor-derived hepatocytes, cholangiocytes and colonic cells, among other epithelial cells (13, 8), after human allogeneic HCT. However, at the same time a significant number of studies failed to detect donor-derived epithelial cells after allogeneic HCT (14-16). Moreover, initial studies claiming to detect donor-derived epithelial cells (or even more plasticity) after HCT were treated with doubt because of methodological limitations. Alternative explanations were given for some of the suspected chimeric events found in the first "positive" studies, such as cell overlapping and/or lymphocyte contamination in the histological sections studied (2).

Despite the initial scepticism and the methodological limitations, more recent studies using strict criteria and examinations of isolated single cells have clearly shown that following allogeneic hematopoietic cell transplantation (HCT) in humans, epithelial cells with donor-derived genotype emerge (17-19). Spyridonidis *et al (*20) applied a three-dimensional analysis on single colon sections from transplanted women after triple stain with donor specific, epithelial-specific and hematopoietic-specific markers, and could clearly show that emergence of epithelial cells with donor-derived genotype after human HCT is a real phenomenon. Tran *et al (*21) and Metaxas *et al* (22) found that isolated buccal epithelial cells obtained by oral scraping from allo-transplanted women, contained the Y-chromosome within their nucleus, as detected by FISH, suggesting that HCT results in generation of individual epithelial cells with donor-derived genotype. To minimize confusion we omit use of the term "plasticity" and "transdifferentiation" in order to describe the above mentioned observations since these terms implicate mechanisms. We prefer to use the term "epithelial chimerism" or more specified tissue chimerism like "myocardial chimerism" when we detect non-hematopoietic cells with donor-derived genotype (2).

The need to understand the possible clinical significance of the findings quickly shifted the interest of research to comprehend the underlying mechanisms of emergence of epithelial chimerism. Understanding of these mechanisms might be helpful to settle in stem cells for the clinical use of tissue repair. Suggested theories include transdifferentiation of hematopoietic cells, generation of epithelial cells from unknown epithelial precursors and/or universal stem cells, or fusion of donor hematopoietic cells with recipient epithelial cells (2). Transdifferentiation is observed in lower vertebrates (23) and in some *in vitro* experiments with adult mammalian cells (24), but has been never clearly and unequivocally demonstrated that such an event could also occur naturally *in vivo*. Krause *et al* (25) demonstrated that a single cell capable of long-term reconstitution of hematopoiesis in lethally irradiated mice was also capable of differentiating into epithelial cells of the liver, lung, gastrointestinal tract and skin making this study the best example of possible plasticity potential of hematopoietic stem cells. However, even this elegant study had some limitations and could not fulfil the criteria for demonstration of plasticity, such as robust detection of epithelial chimerism, documentation of functionality of the chimerical epithelial cells and clonal analysis of the chimerical cells to verify their origin from a hematopoietic stem cell (2). Other researchers suggest that tissue specific progenitors (26, 27) or a universal type of stem cell, like "multipotent adult progenitor cell" (28) or "Very Small Embryonic Like cell" (VSEL) (29), may exist in adult BM. Although these studies showed that these cells can differentiate into all three germ lines in cultures *in vitro* it is not unambiguously shown whether this capacity exist in a significant percentage *in vivo* or is induced mainly through *in vitro* manipulation (2). Another proposed mechanism aiming to explain epithelial chimerism after allogeneic HCT is the fusion of bone-marrow derived stem cells with tissue-specific differentiated cells. Although this mechanism could operate in specific experimental systems, like the liver regeneration through HCT in the FAH–/– transplanted mice (30, 31), this has never been shown in

other tissues. A fusion process of the very primitive VSEL with somatic cells has been implicated in carcinogenesis by forming heterocaryons and selecting for aneuploid cancer cells (32). Whether such a fusion mechanism between transplanted very primitive VSEL cells and recipient epithelial cells contributes to the epithelial chimerism and/or chromosomal instability and secondary neoplasia after HCT is not known. On the other hand, several reports using Cre/lox technology (10, 33) or chromosome analysis suggest that non-fusion mechanism may be implicated in the generation of BM-derived epithelial cells (20, 21).

Recent findings suggest a novel mechanism explaining epithelial chimerism after allogeneic HCT. Jang *et al* (34) found that when murine hematopoietic stem cells are co-cultured with injured liver separated by a barrier, they may convert into liver-like cells. This study suggested that factors contained in the extracts of damaged liver cells may mediate the conversion of the hematopoietic stem cells to hepatocytes. Ratajczak J *et al* were the first to propose microvesicle – mediated horizontal mRNA transfer from donor to recipient cells as a potential underlying mechanism of stem cell plasticity (35). In this study, Ratajczak J *et al* showed that mRNA - containing membrane fragments called microvesicles are released from exponentially growing murine embryonic cells and these cellular fragments may be delivered / fused with hematopoietic progenitor cells and up-regulate in these cells the expression of hematopoietic stem cell markers (35). Similarly, Aliotta *et al* (36) recently demonstrated that the lung-conditioned medium contains microvesicles carrying lung-specific mRNA which are able to enter bone marrow cells in culture and induce in marrow cells lung – specific protein expression.

In general, the mechanism proposed by Ratajczak J *et al* (35) and Aliotta *et al* (36) shares the common feature of different molecule trafficking between cells (37). Broadly the mechanisms describing this trafficking can be classified in three different categories according to the type of material exchanged between the cells: DNA exchange, also known as horizontal gene transfer (HGT) (38-39), RNA or DNA exchange usually done by microvesicles shed from the plasma membrane under certain cell conditions (35, 40, 41) and trogocytosis defined by the uptake of membrane fragments from one cell by another (42, 43). Also, it has been reported the transference of organelles between the cells, such as mitochondria which can rescue the cell from anaerobic metabolism (44, 45). Thus, not only the mechanism described by Ratajczak J and Aliotta *et al*, but also a combination of these recently proposed mechanisms could explain the phenotypic and functional changes in the cells of different tissues after HCT (35, 36). It is possible that the "type" of the delivered genetic material (DNA vs mRNA) may play a role. Whilst mRNA exchange may induce transient / short-term effects in recipient cells, DNA exchange may be responsible for the long-term cellular changes observed. In the case of HGT, DNA released from the hematopoietic-derived donor cells will be transferred, incorporated into the nucleus and eventually expressed by the recipient epithelial cell, ultimately leading to chimerism in individual nuclei (DNA chimerism) and emergence of epithelial cells with donor-derived genotype.

4. GENOMIC INSTABILITY IN EPITHELIUM AFTER ALLOGENEIC HCT

After allogeneic HCT, epithelial tissues become injured through the preparative regimen and are then potentially attacked by allo-reactive T cells. The net effect of these allo-antigeneic reactions is tissue stress and apoptosis which we recognize clinically as GvHD (5). Faber *et al* (6) hypothesized that chronic tissue stress due to interaction of donor-derived lymphocytes with host epithelium in the biological chimeras developed after HCT may cause genomic alterations and therefore analysed epithelial tissues from allogeneic transplanted patients at molecular level. The authors found frequent genomic alterations measured as microsatellite instability in non-neoplastic epithelial tissues of patients who underwent allogeneic HCT. These genomic alterations were found only in allogeneic transplanted patients but not after autologous HCT or intensive chemotherapy, and therefore they suggested that allogeneic reactions after allo-HCT, e.g. GvH reactions, are substantially involved in the mutation process. In subsequent analyses performed in additional 70 patients who underwent allo-HCT, the authors confirmed their previous results by demonstrating genomic instability in non-neoplastic tissues of nearly 50% of the allogeneic transplanted patients, especially in old recipients and those who suffered from chronic GvHD (46). Moreover, this study suggested that occurrence of genomic instability after allogeneic HCT might play a role in the development of secondary neoplasia (46). Elucidating the ultimate mechanisms underlying the genomic instability following allo-antigeneic reactions in the chimeric organism is a major challenge which might have important clinical implications like the protection of the epithelium during GvHD and the prevention of malignant transformation. In the following we propose horizontal gene transfer and illegitimate integration of donor DNA in recipient epithelial cells as the underlying mechanism operating in the occurrence of genomic instability after allogeneic HCT.

5. HORIZONTAL GENE TRANSFER

Horizontal gene transfer (HGT) is a phenomenon in which DNA from one cell (donor cell) is transferred, incorporated and sometimes expressed by another cell (recipient cell). HGT is well described in prokaryotic organisms as a mean for fast adaptation to changing environmental conditions, antibiotic resistance and also as an evolutionary mechanism (47). In eukaryotic organisms HGT has been assumed to be of limited significance, however recent reports have indicated HGT as a potentially important mechanism of genetic material transfer between cells in different clinical settings such as inflammation, cancer and after tissue or organ transplantation (37, 48, 49). It has been suggested that DNA from tumour cells or DNA circulating in the plasma of cancer patients may be taken up by phagocytic cells and these tumour-DNA containing

cells can become a vehicle for cancer dissemination (genometastasis hypothesis) (50).

In the following we review some aspects of genetic material trafficking between cells through HGT, e.g. origin and composition of extracellular genetic material, nucleic acid uptake by cells, fate of the uptaken genetic material within the cell, illegitimate integration of foreign DNA in recipient DNA, and its possible implications to the recipient cell. We propose that HGT and illegitimate integration of donor DNA in recipient epithelial cells may operate in the development of both epithelial chimerism and genomic instability after allogeneic HCT.

6. ORIGIN AND COMPOSITION OF EXTRACELLULAR GENETIC MATERIAL.

Naked DNA has been isolated from plasma of healthy individuals as well as from patients with various clinical conditions like cancer, diabetes, autoimmune diseases, heart infarct and solid organ transplantation (51). Blood plasma possesses high DNA-hydrolysing activity mainly due to the presence of DNAse I which provide the main mechanism of circulating DNA clearance in the organism (52). Nucleic acid isolated from plasma is present in nucleosomes and in complexes with different plasma proteins such as albumin, immunoglobulins and fibronectin and thus is partially protected from nuclease degradation (53-55). It has also been suggested the presence of circulating DNAse inhibitors in cancer patients as a contributing factor for cancer dissemination.

Apoptosis is a well recognized source of DNA in several clinical settings, such as cancer, HCT, extensive burning and solid organ transplantation. The rapid clearance of apoptotic cells is critically important in order to prevent inflammatory responses and damage by cellular components like proteases. Alterations in this critical clearance system that leads to the persistence of apoptotic cells *in situ* may have pathological consequences such as autoimmune reactions (56, 57).

Food is considered to be an important source of foreign DNA. Large amounts of DNA are ingested daily with the food. Some experiments done in mice by Doerfler *et al* have shown that fragments of ingested DNA are not degraded in the gastrointestinal tract and can reach peripheral white blood cells, spleen and liver (58). However, there is no evidence that the ingested DNA could be expressed in different tissues since when mice were fed with a plasmid DNA carrying the green fluorescent protein gene (GFP) no transcription of the GFP gene was found in tissues by RT-PCR (59).

Few groups have determined the presence of circulating RNA. Using highly sensitive methodology, Rykova *et al* found up to 2.5 ng/ml of circulating RNA in the blood of healthy individuals (60). It is interesting to note that Kopreski *et al* also found RNA in plasma of both healthy individuals and patients and that this RNA was intact enough to allow RT-PCR amplification (61). Previous reports have shown that when free RNA is added to blood, the RNA is non-amplifiable, but when RNA is associated with apoptotic bodies, then it remains stable in serum (62). It has also been suggested that DNA and RNA are packaged into separate apoptotic bodies suggesting that the phagosomes of the cell are specialized in DNA or RNA degradation (63, 64).

7. NUCLEIC ACID UPTAKE BY THE CELLS

The mechanisms for nucleic acid uptake by eukaryotic cells are poorly understood. Experimental evidence suggests that there are several mechanisms for nucleic acid uptake by mammalian cells. A receptor mediated transport system of oligonucleotides has been described in keratinocyte cell lines (65). This system is characterized by a fast, irreversible and saturable uptake of oligonucleotides that is not altered by incubation at low temperature. Mild trypsin treatment of the cells and preincubation with polyanions, like heparin and dextran sulphate, resulted in an inhibition of nucleic acid uptake suggesting the presence of plasma membrane proteins involved in the binding and internalization of these oligonucleotides into the cell. Once the oligonucleotides are inside the cell, they fastly accumulate in the nucleus (53, 65).

Phagocytosis of apoptotic bodies by macrophages, dendritic or non professional phagocytic cells can also result in DNA transfer between cells. It is generally assumed that apoptotic bodies, once having been engulfed by surrounding cells, are fused with lysosomes within the cell and then degraded. Holmgren *et al* have shown that apoptotic bodies derived from Epstein Barr Virus (EBV) infected cell line can be engulfed by fibroblasts, monocytes and endothelial cells and the DNA within these apoptotic bodies can be subsequently expressed. They have also shown that the transferred DNA could be propagated providing in this way a selective advantage of the cell which may result in aneuploidy and the accumulation of genetic changes that are necessary for tumor formation (40).

Membrane derived microvesicles (MV) are m - RNA and protein - containing cellular fragments which, in contrast to apoptotic bodies released by dying cells, are released from activated viable cells and are implicated in cell-to-cell communication (35). The mRNA contained in MV is shown to be delivered to several types of cells and to modify the target cell behavior. For instance, MV derived from activated platelets can induce metastasis and angiogenesis in lung cancer (41). Tumor derived MV can also transfer surface determinants and mRNA of tumor cells to monocytes suggesting contribution for tumor spreading in certain types of tumors (66). Embryonic stem cell-derived MV have also been involved in the reprogramming of hematopoietic progenitors via mRNA transfer (35). Recently a Cre-loxP recombination system was developed to study the activation of silent genes following horizontal gene transfer (67).

Recently, Rustom *et al* described the existence of nanotubular structures transiently connecting mammalian

cells. These nanotube structures had a diameter of 50 to 200 nm, can stretch more than several cell diameters in length and can transfer multiprotein complexes, organelles and genetic material between the cells (68). This is a novel type of cell-to-cell communication which may result in a cell fate change. However future studies are needed to evaluate if this type of structure exists within intact tissue and which are the biological consequences of this kind of communication between the cells.

8. FATE OF THE FOREIGN GENETIC MATERIAL WITHIN THE CELL

As we mentioned in the previous section, little is known about the uptake mechanisms of nucleic acids by mammalian cells. Even less is known about the fate of these nucleic acids once inside the cell. After its entry into the cell a large proportion of the foreign DNA is rapidly degraded or diluted among subsequent cell divisions. However some foreign DNA fragments will integrate into chromosomal DNA. Uptake of DNA from different natural sources such as apoptotic bodies, mitochondrial DNA and retrotranspositions events will give rise to *de novo* integration of DNA (69, 70). Relatively little is known about the uptake of DNA into the nucleus itself, since this is not a part of normal cellular physiology. Except during mitosis, molecular access to the nuclear interior is limited to the passage through the nuclear pores. When HeLa cells were permeabilized with digitonin and exposed to double stranded DNA of approximately 1 kb in length, the uptaken DNA was localized in the nucleus. This process seems to be an active transport through the nuclear pore and energy dependent. However, when the same cell line was microinjected with DNA, no intranuclear labeled DNA could be seen after 4 and 24 hours of examination (71). This difference could be due in part to the amount of DNA to which the cells have been exposed and also because digitonin could disrupt insoluble cytoplasmic elements making it easier for the DNA to reach the nuclear pore. Once the DNA is inside the nucleus some fragments could integrate into chromosomal DNA. The integration of foreign DNA is considered to be a low efficiency process with a frequency integration of 1 into 1000 cells but this frequency could vary depending on the cell type and the experimental conditions (72, 73). There are two possible ways that DNA could be integrated into chromosomes: by homology dependent means or by illegitimate integration (74). Homology dependent genome modifications have numerous applications in the field of gene therapy where DNA fragments are used to modify locus with punctual mutations in order to correct genetic defects (75). When illegitimate integration takes places, is not (yet) possible to pre-select neither the genomic site of integration nor the DNA sequence to be integrated, so the resulting foreign DNA-chromosome structure or the biological consequences of this process cannot be predicted. This should be taken in consideration for gene therapy studies since it is likely that local or systemic delivery of DNA will eventually result in illegitimate integration of DNA (76, 77).

Inside the nucleus, foreign extra-chromosomal DNA can be modified before or during the integration process. Several point mutations, deletions and more complex rearrangements such as insertions of genomic DNA have been reported (78-80). It is interesting to note that the amount of integrated DNA depends on the cell type. Human cells integrate foreign DNA in their genomes 30 to 100 times less than rodent cells (81).

A DNA repair-mediated integration process has been proposed as a mechanism for illegitimate DNA integration (82, 83). In this model a genomic lesion will attract the cell repair machinery, so if the nucleus contains some foreign DNA with free ends, this DNA could be used to repair the lesion and become a part of the healing process. This model is supported by the observation that induction of DNA damage by treatment of cells with topoisomerase I and II inhibitors, restriction enzymes, cross-linking agents or UV damage leads to an increase frequency of illegitimate DNA integration (84, 85). Also, a deficiency of mismatch repair enzymes can increase the frequency of illegitimate DNA integration several times when compared with cells with an intact repairing system (86).

Illegitimate DNA integration has been traditionally considered a random process, where foreign DNA can integrate any genomic loci with the same probability. However few data support this concept and it has been shown that not all chromosomes are subject to an equal degree of illegitimate DNA integration (87). Using an insertional mutagenesis model in transgenic mice, the foreign DNA integration frequency was higher in chromosomes 6 and 10 compared with other chromosomes, suggesting that these chromosomes may have particular features that make them more susceptible to integrate foreign DNA (88). Also some cell types like spermatozoa are very selective for illegitimate DNA integration and they can integrate foreign DNA at one or few specific sites, suggesting in this way restrictions and a selective pattern of integration (89).

9. PERSPECTIVE

Continuous research in the field of allogeneic HCT reveals hidden consequences of the unphysiological creation of biological chimeras. The development of epithelial cells with donor - derived genotype and the accumulation of genomic alterations in the epithelial tissues are only two of these recently recognized phenomena occurring after allogeneic HCT. It is unclear whether these two phenomena, which are both presented in the host epithelium, occur independently or are indeed etiologically linked. A possible link between these two phenomena could be provided by HGT. A potential scenario which needs further experimental proof is the following: In the transplantation setting the transplanted and engrafted bone marrow produces continuously hematopoietic cells, like lymphocytes and granulocytes, in the recipient's body. These cells have a limited life span, undergo apoptosis and charge constantly the host environment with donor DNA. It must also be taken in

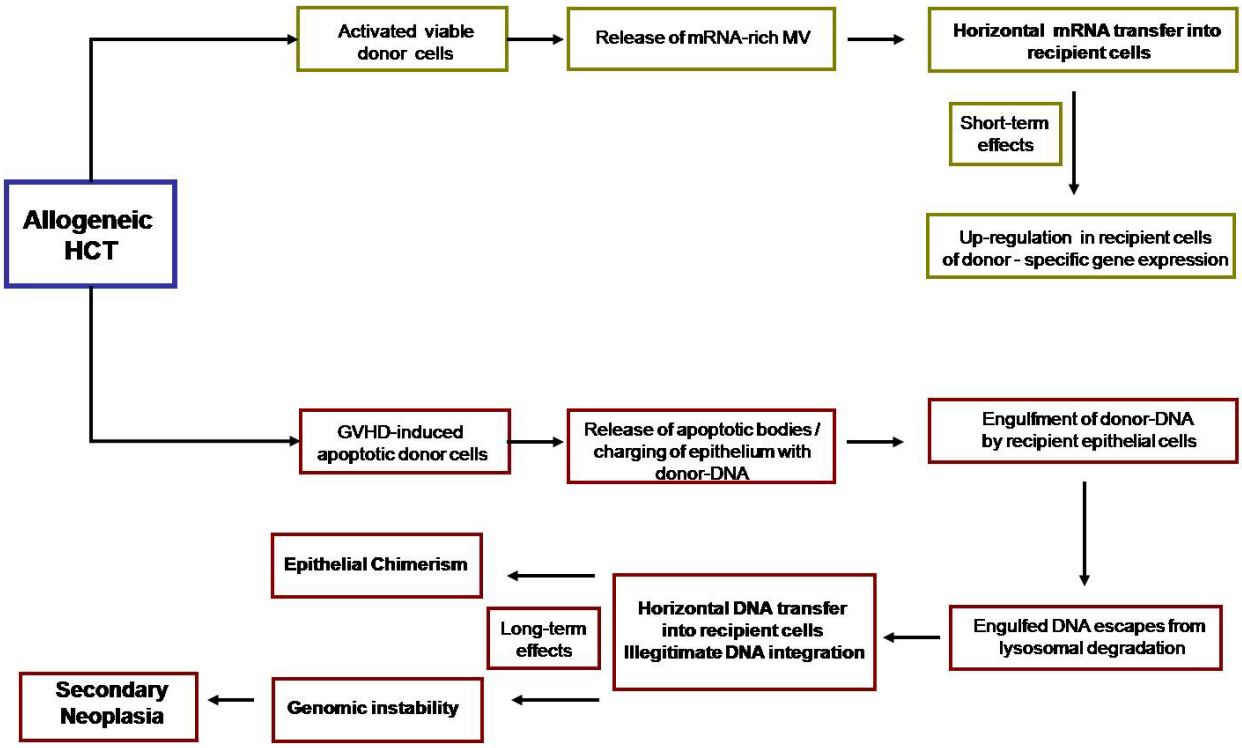

Figure 1. Horizontal gene transfer between donor and recipient cells as a possible mechanism explaining and linking epithelial chimerism and genomic instability after human hematopoietic cell transplantation.

consideration that apoptosis is a hallmark of GvHD resulting in the generation of large amount of apoptotic bodies. The donor-derived DNA apoptotic material may be engulfed by professional and non professional phagocytic cells, fused with lysosomes within the cell and then degraded. However, during GvHD, activated lymphocytes and macrophages generate intracellular reactive oxygen species (ROS) which are shown to severely damage cellular components such as DNA and cellular membranes (90, 91). Inside the lysosomal apparatus, generated ROS along with low molecular weight iron, reducing amino acids (cysteine) and the acidic milieu may destabilize and damage lysosomal membranes and cause lysosomal leakage (92). Also, intracellular ROS may induce oxidative modifications of DNA bases and damage DNA mechanisms that are intrinsically present in cells to avoid foreign DNA integration including DNA-checkpoints such as p53 (90, 93). In this context, the excessive amount of DNA taken up by the recipient cells may overcome their lysosomal degrading capacity and a part of the donor derived DNA may escape from degradation by lysosomes, transported into the nucleus and integrate with the recipient DNA. This "inappropriate" illegitimate integration of donor DNA in epithelial cells may come in light as detection of epithelial cells with donor-derived genotype or as genomic instability in the epithelium (Figure 1).

Taken together, we propose that horizontal gene transfer between donor and recipient cells is very likely to occur after HCT in humans and could be the cornerstone mechanism for the genomic changes occurring in the host epithelium. Lymphocyte-epithelial interactions between the two genetically distinct cell populations in the transplant recipient should be investigated more precisely not only in cellular but also in molecular level. Focusing on the pathways through which these interactions cause genomic changes may bring up novel therapeutic targets for the protection of the epithelium during GvHD and the prevention of malignant transformation.

10. ACKNOWLEDGMENTS

Supported by Landesstiftung Baden Württemberg. We would like to thank our colleagues from the Department of Hematology in Patras University Medical Center, Patras, Greece and the Department of Hematology-Oncology in Albert-Ludwigs University Medical Center, Freiburg, Germany. This work was supported by Landesstiftung Baden Württemberg.

11. REFERENCES

1. MT Little, R Storb: History of haematopoietic stem-cell transplantation. *Nat Rev Cancer* 2, 231-238 (2002)

2. A Spyridonidis, R Zeiser, M Follo, Y Metaxas, J Finke: Stem cell plasticity. The debate begins to clarify. *Stem Cell Rev*, 1, 37-44 (2005)

3. E Copelan: Hematopoietic Stem-Cell Transplantation. *N Eng J Med*, 534, 1813-1826 (2006)

4. SM Lanzkron, MI Collector, SJ Sharkis: Hematopoietic stem cell tracking *in vivo*: a comparison of short- term and long- term repopulating cells. *Blood*, 93, 1916-1921 (1999)

5. R Zeiser, R Marks, H Bertz, J Finke: Immunopathogenesis of acute graft-versus-host disease: implications for novel preventive and therapeutic strategies. *Ann Hematol*, 83, 551-565 (2004)

6. P Faber, P Fish, M Waterhouse, A Schmitt-Graeff, H Bertz, J Finke, A Spyridonidis: Frequent genomic alterations in epithelium measured by microsatellite instability following allogeneic hematopoietic cell transplantation in humans. *Blood*, 107, 3389-3396 (2006)

7. M Korbling, RL Katz, A Khanna, AC Ruifrok, G Rondon, M ALbitar, RE Champlin, Z Estrov: Hepatocytes and epithelial cells of donor origin in recipients of peripheral- blood stem cells. *N Eng J Med*, 346, 738-746 (2002)

8. ND Theise, M Nimmakayalu, R Gardner, PB Illei, G Morgan, L Teperman, O Henegariu, DS Krause: Liver from bone marrow in humans. *Hepatology*, 32, 11-16 (2000)

9. G Ferrari, GC de Angelis, M Coletta, E Paolucci, A Stornaiuolo, G Cossu, F Mavilio: Muscle regeneration by bone marrow- derived myogenic progenitors. *Science*, 279, 1528-1530 (1998)

10. A Ianus, GG Holz, ND Theise, MA Hussain: *In vivo* derivation of glucose-competent pancreatic endocrine cells from marrow without evidence of cell fusion. *J Clin Invest*, 111, 843-850 (2003)

11. E Mezey, KJ Chandross, G Harta, RA Maki, SR McKercher: Turning blood into brain: Cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science*, 290, 1779-1782 (2000)

12. FD Camargo, R Green, Y Capetanaki, KA Jackson, MA Goodell: Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat Med*, 9, 1520-1527 (2003)

13. MR Alison, R Poulsom, R Jeffrey, AP Dhillon, A Quaglia, J Jacob, M Novelli, G Prentice, J Williamson, NA Wrigth: Hepatocytes from nonhepatic adult stem cells. *Nature*, 406, 257 (2000)

14. AJ Wagers, RI Sherwood, JL Christensen, IL Weissman: Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*, 297, 2256-2259 (2002)

15. K Ono, K Yoshihara, H Suzuki, KF Tanaka, T Takii, K Onozaki, M Sawada: Preservation of hematopoietic properties in transplanted bone marrow cells in the brain. *J Neurosci Res*, 72, 503-507 (2003)

16. RF Castro, KA Jackson, MA Goodell, CS Robertson, H Liu, HD Shine: Failure of bone marrow cells to transdifferentiate into neural cells *in vivo*. *Science*, 297, 1299 (2002)

17. CR Cogle, TA Yachnis, ED Laywell, DS Zander, JR Wingard, DA Steindler, EW Scott: Bone marrow transdifferentiation in brain after transplantation: a retrospective study. *Lancet*, 363, 1432-1437 (2004)

18. J Mattsson, J Monika, A Wernerson, H Moustapha: Lung Epithelial Cells and Type II Pneumocytes of Donor Origin After Allogeneic Hematopoietic Stem Cell Transplantation. *Transplantation*, 78, 154-157 (2004)

19. R Okamoto, T Yajima, M Yamazaki, T Kanai, M Mukai, S Okamoto, Y Ikeda, T Hibi, J Inazawa, M Watanabe: Damaged epithelia regenerated by bone marrow–derived cells in the human gastrointestinal tract. *Nat Med*, 8, 1011-1017 (2002)

20. A Spyridonidis, A Schmitt-Graeff, T Tomann, A Dwenger, M Follo, D Behringer, J Finke: Epithelial tissue chimerism after Human Hematopoietic Cell Transplantation is a real phenomenon. *Am J Pathol*, 164, 1147-1155 (2004)

21. SD Tran, SR Pillemer, A Dutra, AJ Barrett, MJ Brownstein, S Key, E Park, RA Leakan, A Kingman, KM Yamada, BJ Baum, E Mezey: Differentiation of human bone marrow-derived cells into buccal epithelial cells *in vivo*: a molecular analytical study. *Lancet*, 361, 1084-1088 (2003)

22. Y Metaxas, R Zeiser, A Schmitt-Graeff, M Waterhouse, P Faber, M Follo, H Bertz, J Finke, A Spyridonidis: Human hematopoietic transplantation results in generation of donor derived epithelial cells. *Leukemia*, 19, 1287-1289 (2005)

23. JP Brockes, A Kumar: Plasticity and reprogramming of differentiated cells in amphibian regeneration. *Nat Rev Mol Cell Biol*, 3, 566-574 (2002)

24. K Takahashi, S Yamanaka: Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*, 126, 663-676 (2006)

25. DS Krause, ND Theise, MI Collector, O Henegariu, S Hwang, R Gardner, S Neutzel, SJ Sharkis: Multi-organ, multilineage engraftment by a single bone marrow- derived stem cell. *Cell*, 105, 369-377 (2001)

26. I Avital, D Inderbitzin, A Takeshi, DB Tyan, AH Cohen, C Ferraresso, J Rozga, WS Arnaout, AA Demetriou: Isolation, Characterization, and Transplantation of Bone Marrow-Derived Hepatocyte Stem Cells. *Biochem Biophys Res Commun*, 288, 156-164 (2001)

27. MZ Ratajczak, M Kucia, R Reca, M Majka, A Janowska-Wierczorek, J Ratajczak: Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells 'hide out' in the bone marrow. *Leukemia*, 18, 29-40 (2004)

28. Y Jiang, BN Jahagirdar, RL Reinhardt, RE Schwartz, CD Keene, XR Ortiz-Gonzalez, M Reyes, T Lenvik, T Lund, M Blackstad, J Du, S Aldrich, A Lisberg, WC Low, DA Largaespada, CM Verfaillie:

Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, 418, 41-49 (2002)

29. M Kucia, M Halasa, M Wysoczynski, M Baskiewicz-Masiuk, S Moldenhawer, E Zuba-Surma, R Czajka, W Wojakowski, B Machalinski, MR Ratajczak: Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood – preliminary report. *Leukemia*, 21, 297-303 (2007)

30. G Vassilopoulos, PR Wang, DW Russell: Transplanted bone marrow regenerates liver by cell fusion. *Nature*, 422, 901-904 (2003)

31. Xin Wang, H Willenbring, Y Akkari, Y Torimaru, M Foster, M Al-Dhalimy, E Lagasse, M Finegold, S Olson, M Grompe: Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature*, 422, (2003)

32. MZ. Ratajczak, EK. Zuba-Surma, M Wysoczynski, W Wan, J Ratajczak, W Wojakowski, M Kucia: Hunt for pluripotent stem cell – Regenerative medicine search for almighty cell. *Journal of Autoimmunity*, 30, 151-162 (2008)

33. RG Harris, EL Herzog, EM Bruscia, JE Grove, JS Van Arnam, DS Krause: Lack of fusion requirement for development of bone marrow- derived epithelia. *Science*, 305, 90-93 (2004)

34. YY Yang, MI Collector, SB Baylin, AM Diehl, SJ Sharkis: Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol*, 6, 532-539 (2004)

35. J Ratajczak, K Miekus, M Kucia, J Zhang, R Reca, P Dvorak, MZ Ratajczak: Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia*, 20, 847-856 (2006)

36. Aliotta JM, Sanchez-Guijo FM, Dooner GJ, Johnson KW, Dooner MS, Greer KA, Greer D, Pimentel J, Kolankiewicz LM, Puente N, Faradyan S, Ferland P, Bearer EL, Passero MA, Adedi M, Colvin GA, Quesenberry PJ: Alteration of marrow cell gene expression, protein production, and engraftment into lung by lung-derived microvesicles: a novel mechanism for phenotype modulation. *Stem cells,* 25, 2245-56 (2007)

37. J Andersson: Lateral gene transfer in eukaryotes. *Cell Mol Life Sci*, 62, 1182-1197 (2005)

38. A Bergsmerdh, A Szeles, M Henriksson, A Bratt, M Folkman, A Spetz, L Holmgren: Horizontal transfer of oncogenes by the uptake of apoptotic bodies. *Proc Nat Acad Sci*, 98, 6407-6411 (2001)

39. J Raymond, R Blakenship: Horizontal gene transfer in eukaryotic algal evolution. *Proc Nat Acad Sci*, 100, 7419-7420 (2003)

40. L Holmgren, A Szeles, E Rajnavolgyi, J Folkman, G Klein, I Ernberg, K Falk: Horizontal transfer of DNA by the uptake of apoptotic bodies. *Blood*, 93, 3956-3963 (1999)

41. A Janowska-Wieczorek, M Wysoczynski, J Kijowski, L Marquez-Curtis, B Machalinski, J Ratajczak: Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int J Cancer*, 113, 752-760 (2005)

42. E Joly, D Hudrisier: What is trogocytosis and what is the purpose? .*Nat Immunol*, 4, 815 (2003)

43. J LeMaoult, J Caumartin, M Daouya, B Favier, S Le Rond, A Gonzalez, E Carosella: Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. *Blood*, 109, 2040-2048 (2007)

44. A Barrientos, C Moraes: Simultaneous transfer of mitochondrial DNA and single chromosomes in somatic cells: a novel approach for the study of defects in nuclear-mitochondrial communication. *Hum Mol Genet*, 7, 1801-1808 (1998)

45. J Spees, S Olson, M Whitney, D Prockop: Mithocondrial transfer between cells can rescue aerobic respiration. *Proc Nat Acad Sci*, 103, 1283-1288 (2006)

46. M Themeli, L Petrikkos, M Waterhouse, H Bertz, N Zoumbos, J Finke, A Spyridonidis: Alloantigenic reactions after human haematopoietic cell transplantation induce genomic alterations in epithelium: a confirmation *in vivo* and *in vitro* study and implications to secondary neoplasia. *EBMT*, (2008).

47. CG Kurland, B Candback, O Berg: Horizontal gene transfer: a critical view. *Proc Nat Acad Sci*, 100, 9658-9662 (2003)

48. KS Tysnes, R Bjerkiv: Cancer initiation and progression: Involvement of stem cells and the microenvironment. *Biochim Biophys Acta*, 1775, 283-297 (2007)

49. R Bjerkvig, KS Tysnes, J Aboody, AJ Najbauer, J Terzis: The origin of the cancer stem cell: current controversies and new insights. *Nat Rev Cancer*, 5, 899-904 (2005)

50. D Olmo-Garcia, DC Garcia-Olmo, J Ontanon, E Martinez, M Vallejo: Tumor DNA circulating in the plasma may play a role in metastasis. The hypotesis of the genometastasis. *Histol Histopathol*, 14, 1159-1164 (1999)

51. M Stroun, P Maurice, V Vasioukhin, J Lyuatey, C Lederrey: The origin and mechanism of circulating DNA. *Ann NY Acad Sci*, 906, 161-168 (2000)

52. WS Prince, DL Baker, AH Dodge, AE Ahmed, RW Chestnut: Pharmacodynamics of recombinant human DNase I in serum. *Clin Exp Immunol*, 113, 289-296 (1998)

53. BP Chelobanov, PP Laktionov, W Vlassov: Proteins involved in binding and cellular uptake of nucleic acids. *Biochemistry*, 71, 583-596 (2006)

54. EY Rykova, LV Paultova, LA Yakubov, VN Karamishev, W Vlassov: Serum immunoglobulins interact with oligonucleotides. *FEBS lett*, 344, 96-98 (1994)

55. TE Skvorstova, EY Rykova, TN Tamkovich, OE Bryzgunova, TE Skvorstova: Cell-free and cell-bound circulating DNA in breast tumors: DNA quantification and analysis of tumor related gene methylation. *Br J Cancer*, 94, 1492-1495 (2006)

56. PM Rumore, CR Steinman: Endogenous circulating DNA in systemic lupus erythematosus. Ocurrence as multimeric complexes bound to histone. *J Clin Invest*, 86, 69-74 (1990)

57. TM Yeh, HC Chang, CC Liang, JJ Wu, MF Liu: Deoxyribonuclease-inhibitory antibodies in systemic lupus erythematosus. *J Biomed Sci*, 10, 544-551 (2003)

58. W Doerfler, U Hohlweg, K Muller, R Remus, H Heller, J Hertz: Foreign DNA integration-Pertubations of the Genome-Oncogenesis. *Ann NY Acad Sci*, 906, 276-288 (2000)

59. K Kawabata, Y Takakura, M Hashida: The fate of plasmid DNA after intravenous injection in mice: involvement of scavenger receptors in its hepatic uptake. *Pharm Res*, 12, 825-830 (1995)

60. EY Rykova, OE Bryzgunova, TE Skvortsova, TN Tamkovich, IN Senin: Concentrations of circulating RNA from healthy donors and cancer patients estimated by different methods. *Ann NY Acad Sci*, 1075, 328-333 (2006)

61. MS Kopreski, FA Benko, LW Kwak, CD Gocke: Detection of tumor messenger RNA in the serum of patients with malignant melanoma. *Clin Cancer Res*, 5, 1961-1965 (1999)

62. NB Tsui, EK Ng, YM Lo: Stability of endogenous and added RNA in blood specimens, serum and plasma. *Clin Chem*, 48, 1647-1653 (2002)

63. DO Hasselman, G Rappl, W Tilgen, U Reinhold: Extracellular tyrosinase mRNA within apoptotic bodies is protected from degradation in human serum. *Clin Chem*, 47, 1488-1489 (2001)

64. HD Halica, E Bedner, Z Darzynkiewicz: Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. *Exp Cell Res*, 260, 248-256 (2000)

65. P Laktionov, JE Dazzard, E Vives, E Rykova, J Piette: Characterisation of membrane oligonucleotide-binding proteins and oligonucleotide uptake in keratinocytes. *Nucleic Acids Res*, 27, 2315-2324 (1999)

66. M Baj-Kryworzeka, R Szatanek, K Weglarczyk, J Baran, B Urbanowicz, P Branski, M Ratajczak, M Zembala: Tumor-derived microvesicles carry several surface determinants and mRNA of tumor cells and transfer some of these determinants to monocytes. *Cancer Immunol Immunother*, 55, 808-818 (2006)

67. A Bergsmedh, J Ehnfors, A Spetz, L Holmgren: A Cre-lox P based system for studying horizontal gene transfer. *FEBS lett*, 581, 2943-2946 (2007)

68. A Rustom, R Saffrich, I Markovic, P Walther, HH Gerdes: Nanotubular highways for intracellular organelle transport. *Science*, 303, 1007-1010 (2004)

69. C Esnault, J Maestre, T Heidmann: Human LINE retrotransposons generate processed pseudogenes. *Nat Genet*, 24, 363-367 (2000)

70. DE Berg, MM Howe: Mobile DNA. *American society for Microbiology;* Washington, DC *(*1989)

71. JE Hagstrom, JJ Ludtke, MC Bassik, MG Sebestyen, SA Adam, JA Wolff: Nuclear import of DNA in digitonin-permeabilized cells. *J Cell Sci*, 110, 2323-2331 (1997)

72. C Gorman, R Padmanabhan, BH Howard: High efficiency DNA-mediated transformation of primate cells. *Science*, 221, 551-553 (1983)

73. JE Stauton, DT Weaver: Scid cells efficiently integrate hairpin and linear DNA substrates. *Mol Cell Biol*, 14, 3876-3883 (1994)

74. H Wurtele, KCE Little, P Chartrand: Illegitimate DNA integration in mammalian cells. *Gene Ther*, 10, 1791-1799 (2003)

75. KK Gonez: Application of SFHR to gene therapy of monogenic disorders. *Gene Ther*, 9, 691-694 (2002)

76. BJ Ledwith: Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. *Intervirology*, 43, 258-272 (2000)

77. S Manam: Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery methods on integration into host DNA. *Intervirology*, 43, 273-281 (2000)

78. JH Wilson, PB Berget, JM Pipas: Somatic cell efficiently join unrelated DNA segments end-to-end. *Mol Cell Biol*, 2, 1258-1269 (1982)

79. MP Calos, JS Lebkowski, MR Botchan: High mutation frequency in DNA transfected into mammalian cells. *Proc Nat Acad Sci*, 80, 3015-3019 (1983)

80. CT Wake: How damaged is the biologically active subpopulation of transfected DNA. *Mol Cell Biol*, 4, 387-398 (1984)

81. JH Hoeijmakers, H Odijk, A Westerveld: Differences between rodent and human cell lines in the amount of integrated DNA after transfection. *Exp Cell Res*, 169, 111-119 (1987)

82. C Chen, LA Chasin: Cointegration of DNA molecules introduced into mammalian cells by electroporation. *Somat Cell Mol Genet*, 24, 249-256 (1998)

83. S Kato, RA ANderson, RD Camerini-Otero RD: Foreign DNA introduced by calcium phosphate is integrated into repetitive DNA elements of the mouse L cell genome. *Mol Cell Biol*, 6, 1787-1795 (1986)

84. K Fujimaki: DNA topoisomerase II inhibitors enhance random integration of transfected vectors into human chromosomes. *Somat Cell Mol Genet*, 22, 279-290 (1996)

85. G Spivak, AK Ganesan, PC Hanawalt: Enhanced trasnformation of human cells by UV-irradiated pSV2 plasmids. *Mol Cell Biol*, 4, 1169-1171 (1984)

86. CT Lin: Suppression of gene amplification and chromosomal DNA integration by the DNA mismatch repair system. *Nucleic Acids Res*, 29, 3304-3310 (2001)

87. G Dellaire, P Chartrand: Direct evidence that transgene integration is random in murine cells, implying that naturally occurring double strand breaks may be distributed similarly in the genome. *Radiat Res*, 149, 325-329 (1998)

88. T Rijerks, A Peetz, U Ruther: Insertional mutagenesis in transgenic mice. *Transgenic Res*, 3, 203-215 (1994)

89. AR Magnano: Sperm/DNA interaction: integration of foreign DNA sequences in the mouse genome. *J Reprod Immunol*, 41, 187-196 (1998)

90. H Wiseman and B Halliwell: Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J*, 313, 17-29 (1996)

91. Pelicano H, Carney D and Huang P: ROS stress in cancer cells and therapeutic implications. *Drug Resistance Updates*, 7, 97-110 (2004)

92. K Kagedal, U Johansson, K Ollinger: The lysosomal cathepsin D mediates apoptosis induced by oxidative stress. FASEB J, 15, 1592-1594 (2001)

93. PA Cerutti: Oxy-radicals and cancer. *Lancet*, 344, 862-3 (1994)

Abbreviations: BM: bone marrow, GvHD: graft versus host disease, HCT: hematopoietic cell transplantation, HSC: hematopoietic stem cells, HGT: horizontal gene transfer, MV: microvesicles

Key Words: Hematopoietic Cell Transplantation, Horizontal Gene Transfer, Epithelial Chimerism, Genomic Instability, Review

Send correspondence to: Alexandros Spyridonidis, Division of Hematology, BMT Unit, University of Patras Medical Center, 26500 Rio / Patras, Greece, Tel: 30-2610-999247, Fax: 30-2610-993950, E-mail: spyridonidis@med.upatras.gr

http://www.bioscience.org/current/vol14.htm