Published in final edited form as:
Exp Hematol. 2010 July ; 38(7): 581–592. doi:10.1016/j.exphem.2010.03.021.

Stem cell plasticity revisited: The continuum marrow model and phenotypic changes mediated by microvesicles

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Abstract

The phenotype of marrow hematopoietic stem cells is determined by cell cycle state and microvesicle entry into the stem cells. The stem cell population is continually changing based on cell cycle transit and thus can only be defined on a population basis. Purification of marrow stem cells only addresses the heterogeneity of these populations. When whole marrow is studied, the long-term repopulating stem cells are in active cell cycle. However, with some variability, when highly purified stem cells are studied, the cells appear to be dormant. Thus, the study of purified stem cells is intrinsically misleading. Tissue-derived microvesicles enhanced by injury effect the phenotype of different cell classes. We propose that previously described stem cell plasticity is due to microvesicle modulation. We further propose a stem cell population model in which the individual cell phenotypes continually changes, but the population phenotype is relatively stable. This, in turn, is modulated by microvesicle and microenvironmental influences.

We and others have observed that marrow cell populations are intrinsically heterogeneous and continually changing. This phenotypic lability extends to the capacity of marrow cells to assume the phenotype of other hematopoietic cells or non-hematopoietic cells and appears to be tightly linked to the cell cycle status of the marrow stem cell.

The continuum model and cell cycle: Intrahematopoietic plasticity

All proliferating cell populations are intrinsically heterogeneous and must continually change phenotype as they progress through cell cycle. Thus, a proliferating population can only be defined on a population basis; clonal studies will only address the degree of heterogeneity of a stem cell population. These concepts were elegantly addressed by Till, McCulloch and Siminovitch in the 1960's when they compared the nature of colony-forming unit spleen (1), the first described stem cell, to radioisotopes (2). An isotope has a very predictable half-life. However, the individual nuclei which compose it have markedly varied half-lives, making

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Conflict of Interest Disclosure:
No financial interest/relationships with financial interest relating to the topic of this article have been declared.

them totally heterogeneous. This is a reasonable view of the nature of adult marrow stem cells; they can only be appropriately defined on a population basis.

A number of studies from our laboratory have shown that the phenotype of the lineage negative rhodamine low Hoechst low (and to a lesser extent the lineage negative Sca-1+) stem cell continuously changes, in a reversible fashion, with cell cycle passage (3–16). Characteristics studied have included short and long-term engraftment into lethally irradiated mice, progenitor numbers, differentiation into granulocytes and megakaryocytes, expression of adhesion protein and cytokine receptor genes, global gene expression, expression of cell cycle genes, capacity to convert to pulmonary epithelial cells and, most recently, the capacity to take up microvesicles. These characteristics vary with cycle phase and are reversible (or at least continue to modulate). These observations led to a continuum theory of stem cell biology in which the phenotype of the adult marrow stem cell is continuously changing based, at least in part, on the cell cycle position of the stem cell (17–28). The applicability of this model to normal steady-state hematopoiesis depends on the assumption that the adult marrow stem cell is an actively cycling cell.

Cell cycle status of marrow stem cells

The extant literature on this point is discordant, with the general consensus being that the adult marrow stem cell is “dormant” or quiescent, but with some reports indicating that it is an actively cycling cell. The colony-forming unit spleen (CFU-S) the original clonal stem cell assay (1) was extensively studied and it was generally found to be relatively quiescent with S phase values of 10% or less (29–33). A number of studies showed higher S-phase values for CFU-S, ranging from 16 to 48% (34–45). Our own work showed varying results from no killing with hydroxyurea or tritiated thymidine to killing rates of up to 25% (45). The work by Necas and Znojil (46) is particularly informative. They determined the number of CFU-S and the fraction synthesizing DNA in individual normal mice of several inbred strains and the data obtained over a period of five years was subjected to analysis of variance. Large differences were shown to exist in the number of CFU-S in the femoral bone marrow of individual mice measured on the same day. These differences were greater if measurements were performed on different days. The fraction of DNA synthesizing CFU-S was on average 30% in these normal mice, but the range of measurements on both the same and different days was 0% to 60%. The authors measured CFU-S from day seven to day 12 and found similar results. This work led to a proposal that there may be “bursts “of CFU-S proliferation over time, not on a circadian basis, but rather stochastic in nature.

A major focus of more recent studies of marrow renewal stem cells has been on highly purified marrow stem cells. In general, marrow is depleted of differentiated cells using differentiated cell-specific antibodies to surface epitopes and magnetic bead separation. This is followed by staining of lineage negative cells for stem cell-related surface antigens and separation by fluorescent activated cell sorting (FACS) (47–67). Studies on purified stem cells have given different views of the cell cycle status of the primitive marrow stem cells. Work by Bradford and colleagues (68), confirmed by Cheshire and colleagues (56) and Pang and colleagues (69), suggested that primitive stem cells were a continuously cycling population. Work on LT-HSC done by Fleming and colleagues (70) suggested that over 20% of these cells were in cell cycle at isolation. Cheshire and colleagues (56) proposed that the population is continuously in cycle and transits cycle fairly rapidly with 50% of LT-HSC showing BrdU incorporation by six days. They estimated that 8% of stem cells entered cycle each day in *in vivo* experiments, while other work proposed a stem cell turnover time of 154 days. This latter study by Wilson and colleagues (71) actually produced *in vivo* BrdU data more consistent with a rapid turnover, but explained this by supposed BrdU toxicity to hematopoietic cells with secondary effects.

However, carefully conducted experiments in the Cheshire studies (56) did not show any BrdU toxicity.

Studies on the marrow stem cell "side population" indicated that S/G2/M cells had the same long-term repopulating capacity as G0 cells (72). Other work using a variety of approaches on different stem cell populations all indicates that a percentage were in S/G2/M at the time of interrogation (34–46,56,68–70,73). Our own studies (74), employing Hoechst/Pyronin separations, have shown that the cell which gives long-term marrow repopulation in whole unseparated marrow is, in fact, actively cycling. While, with a great deal of variation, the purified LT-HSC, as described by Weissman and colleagues, had a small percentage (to none) of stem cells in S/G2/M.

There are several important implications of these results. First, the stem cells purified by antibody-epitope selection are not representative of the stem cells in whole marrow. The epitope selected cells represent specific and relatively rare subsets of stem cells which exclude proliferating and other stem cells from consideration. While the characteristics of a particular epitope-defined stem cells may exist at one point in time or cycle, these characteristics do not persist as that particular cell will continue to change its characteristics eventually returning to its original phenotype. Thus, a G0 Lin-/Sca-1+/c-kit+/slam+ cell may be present at one time in G0, but later, perhaps a few hours into G1, its phenotype may be that of a megakaryocyte progenitor or even a monocyte. Fluxes of phenotypes are obligatory if the cell is in active cell cycle. The overall description of this situation is a stable stem cell population in which the individual entities (or cells) are continually changing, but the whole maintains its general aspect. This is very similar to the radioisotope situation described above. The challenge in the stem cell field is to define the total stem cell population, i.e. those cells which maintain a potential to assume the characteristics of a long-term multipotent engrafting stem cell. One might start with all cells that maintain a capacity for proliferation. This would only exclude anucleated erythrocytes and mature polymorphonuclear granulocytes. The true stem cell population might only be the lineage negative cells, but this remains speculation. We propose here that marrow hematopoietic repopulating stem cells are actively cycling and continuously changing. The classically recognized LT-HSC or LT-HSC-slam phenotypes are simply subpopulations of the true stem cell population. This population contains multiple stem/progenitor cells and possibly differentiated cell phenotypes previously placed in a hierarchy. Thus, there is impressive plasticity within the hematopoietic marrow system. A conceptual model is resented in Figure 1.

Stem cell plasticity: The marrow to non-hematopoietic variety

The capacity of murine marrow cells to form non-hematopoietic cells and tissues after transplantation into irradiated mice was termed "stem cell plasticity". The classic proof of principle came from the studies on the Fumarylacetoacetate hydroxylase (FAH)-deficient mouse; a mouse afflicted with a fatal tyrosinemia (75). This can be controlled with administration of the drug NTBC. Repeated withdrawal of the drug provides both injury and selection. In FAH negative mice transplanted with B-galactosidase-positive transgenic marrow, and subjected to repeated withdrawal of NTBC, large areas of diseased hepatic tissue were replaced with normal β-galactosidase-positive donor-derived hepatocytes. In addition, some mice were cured of this fatal disease. Furthermore, very convincing studies have shown that these marrow-to-hepatocyte conversions are due to cell fusion (76). A large number of subsequent studies have shown that host tissue, usually under injury circumstances, can be partially replaced by cells derived from transplanted marrow. This led to the "stem cell plasticity" controversy, a rather meaningless exercise, which we have previously addressed in a perspective titled "Ignoratio Elenchi" (irrelevant conclusions or red herrings) (77). Proposals were made that for results in this area of investigation to be taken seriously, they had to be

"robust", they had to be on a clonal basis (which only shows heterogeneity), they had to show function (which was never adequately defined) and the biggest red herring of all, they couldn't involve cell fusion. Why this latter point became an issue is unclear, but it, unfortunately, became a major negative feature of grant and manuscript reviews. There were a few "negative" studies which appeared to be designed to obtain negative results and which represented marginal science.

Nonetheless, there is now little question that after marrow transplantation, cells can be found in many different tissues, lung and liver being prominent here. These cells have defining characteristics of the specific tissue under consideration but also carry markers indicating origin from the transplanted marrow cells. In some instances, cell fusion may have been involved but in others it was not. A summary of some of this early work showing marrow conversions to liver, lung and skeletal muscle is presented in Table 1.

In continuing studies, virtually every tissue in the body has been found to be subject to marrow conversions or "stem cell plasticity". There have been over 30 articles on marrow-to-lung conversions and, while the percent conversions varied widely, all studies have demonstrated this. Many studies have also addressed whether cell fusion was the mechanism underlying the observed plasticity. A summary of some of these is presented in Tables 2 and 3.

Ogawa and colleagues (96,97) have extended these studies by publishing observations that hematopoietic marrow cells were the origin of fibroblasts and myofibroblasts, which can be found in many tissues, including intestine, skin, liver and lung. In addition, a number of the plasticity studies have shown function.

Marrow to lung studies in plasticity

We have focused on the capacity of transplanted murine marrow cells to convert to pulmonary epithelial cells. We initially studied the capacity of engrafted marrow cells to convert to pulmonary epithelial cells in a lethally irradiated mouse model (87). In these studies, we saw a wide stochastic variation in conversion rates but always saw conversions. Using green fluorescent protein (GFP) or the Y chromosome (in gender-mismatched transplants) to track transplanted cells, the percentage of bone marrow-derived CD45 negative and cytokeratin positive or prosurfactant B positive cells in the lungs transplanted mice varied from 0% in non-irradiated mice to 1.17–18.9% in irradiated mice. The variations seen in irradiated mice depended upon the dose of irradiation, with increasing conversions rates with increasing levels of host irradiation. This latter also correlated directly with bone marrow engraftment levels. Other variables which influenced plasticity were the marrow subpopulation infused. Our initial studies showed that the marrow cells which led to conversions were c-kit+, Sca-1+ and lineage negative. c-kit-, Sca-1- and lineage positive cells did not significantly engraft in the lung. Treatment of engrafted host mice with G-CSF also increased the conversion rates of marrow to lung cells, presumably on the basis of stem cell mobilization. Further studies indicated that treatment of the marrow cells prior to infusion with the cytokines interleukin-3 (IL-3), IL-6, IL-11 and steel factor markedly influenced marrow-to-lung conversions (98). This correlated with cell cycle progression of the marrow cells *in vitro* and peak conversion rates of GFP positive marrow cells to lung cells were seen at the G1/S interface. Here, we saw a three-fold increase in cells assuming a non-hematopoietic or pulmonary epithelial cell phenotype. This increase was no longer seen in late S/G2. These data indicated that engrafted marrow cells were capable of converting to significant numbers of pulmonary epithelial cells in the irradiated mouse and suggested that radiation-induced lung injury might be important in this process. This work is summarized in Figure 2.

Mechanisms underlying marrow conversions to pulmonary epithelial cells or, more accurately, the presence of lung cells with marrow markers after marrow transplantation: the role of microvesicles

Microvesicle information transfer

Our studies (which will be outlined in detail below) have indicated to us that transfer of cell-derived microvesicles between cells may underlie much of the previously described “stem cell plasticity”. The exact nature of and nomenclature for microvesicles is still evolving. Particles derived from cells, especially injured cells, have been described repeatedly. Small membrane-enclosed vesicles from platelets or red blood cells were first considered to represent cellular junk and largely dismissed as having little biologic significance. Subsequently, membrane-bound particles have been described as originating from mast cells (99), dendritic cells (100), tumor cells (101), reticulocytes (102), epithelial cells (103), B cells (104) and neural cells (105). In fact, it is now apparent that these vesicles probably can be derived from virtually all cell types in the body. Membrane-enclosed vesicles derived from a wide variety of cells have been shown to affect the phenotype of putative target cells under different conditions. Different terms have been used to describe these cellular-derived membrane-enclosed entities, including exosomes (106), microvesicles (107), ectosomes (108), membrane particles (109) exosome-like particles (110) and apoptotic vesicles (111). Vesicles have been characterized by size, density in a sucrose gradient, electron microscopy, sedimentation by ultracentrifugation, lipid composition, main protein markers and intracellular origin (112). Exosomes are 50–80 nm in diameter, endocytic in origin and released into the environment during fusion of multivesiclular bodies with plasma membranes. Microvesicles have been described as being 100nm-1um in diameter and released from surface membranes during membrane blebbing in a calcium flux and calpain-dependent manner. As noted by Théry and colleagues (112), in practice, all vesicle preparations are heterogeneous with different protocols allowing enrichment of one type over another. We have studied vesicles sedimented at 100,000g by ultracentrifugation, which would include both exosomes and microvesicles as classically described, and have found that the mode of electron microscopic tissue preparation changed the morphology dramatically. Cup-shaped vesicles can be seen with one approach and irregularly-shaped and electron dense vesicles with another approach. We will use the generic term “microvesicle” to encompass these populations of vesicles, realizing the heterogeneity of most reported vesicle populations. The evolution of microvesicles from different cell populations is influenced by hypoxia, shear stress, irradiation, chemotherapy, cytokines and different drugs such as Acetaminophen (hepatocytes). A particular focus recently has been on the capacity of microvesicles to influence the phenotype of neighboring cells in other tissues. They have been found to transfer CD41, integrins and CXCR4 (111,113–115) as well as HIV and prions (116,117) between cells. Embryonic stem cell-derived microvesicles have been reported to reprogram hematopoietic stem/progenitor cells via the horizontal transfer of mRNA and protein (118). Similarly, tumor-derived microvesicles, which carry several surface determinants and mRNA, can transfer some of these determinants to monocytes (113). Apoptotic bodies from irradiated Epstein-Barr Virus (EBV)-carrying cell lines have been shown to transfer DNA to a variety of co-cultured cells by integrating copies of EBV, resulting in expression of EBV-encoded genes EBER and EBNAI in recipient cells at high copy number (119). Extracts from T lymphocytes containing transcription factor complexes can induce fibroblasts to express lymphoid genes (120). In addition, endothelial cells exposed to microvesicles derived from endothelial progenitor cells form capillary-like structures both *in vitro* and *in vivo* (121). It is of particular interest that previously-described endothelial progenitor cells may, in fact, represent mononuclear cells which have consumed platelet-derived microvesicles (122). All of these studies indicate a capacity of microvesicles to alter the phenotype of “target” cells toward the phenotype of the microvesicle producing cell.

Microvesicles and marrow to lung conversions

Jang and colleagues (123) cultured hematopoietic stem cells across from damaged liver cells but separated from them by a cell impermeable membrane and demonstrated that the marrow cells expressed genes specific for hepatocyte, such as albumin. This was interpreted as humoral induction of differentiation. These findings prompted our own studies which indicated that it might have represented microvesicle induction of phenotype change (124). Accordingly, we studied marrow cells cultured across from murine lung cells which had been exposed to 0, 500 or 1200 cGy irradiation from three hours to 14 days previously. We then assessed the marrow cells for expression of pulmonary epithelial cell-specific mRNA. Our studies indicated that high levels of expression of clara cell specific protein, surfactant C and surfactant B were seen when marrow cells were exposed for 48 hours or 7 days opposite murine lungs. The highest levels were seen when lungs from mice exposed to 500 cGy five days previously were co-cultured with marrow. The basic culture system is shown in Figure 3.

Further work here showed that cell-free conditioned media (CM) from lung, irradiated or not, would induce pulmonary epithelial cell-specific mRNA production in marrow cells and that the inducing principle was present in the pellet of ultracentrifuged (100,000 g) CM. The pellet contained large numbers of microvesicles, as defined by electron microscopy. There were numerous 100–250 nm membrane-bound vesicles; although, in different experiments, smaller vesicles were also seen. These microvesicles could be stained with the supravital membrane dye PKH26 (red fluorescence) and the supravital cytoplasmic dye CFSE (green fluorescence) and then separated and purified as red/green events by FACS. These fluorescent-labeled microvesicles then were incubated with marrow cells and a minority of the marrow cells took up the microvesicles. Marrow cells loaded with microvesicles are shown in Figure 4 along with electron microscopic images of these microvesicles.

Further work isolating marrow cells which had taken up fluorescent microvesicles by FACS and then determining expression of pulmonary epithelial cell-specific mRNAs showed that only marrow cells which had taken up the microvesicles expressed the pulmonary epithelial cell-specific mRNA. Co-cultured marrow cells were shown to express prosurfactant B protein 21 days after a seven day exposure to irradiated lung fragments. Functional effects of marrow cells co-cultured with irradiated lung cells for 7 days were seen. These cells gave higher levels of prosurfactant C positive donor cells in host lungs after transplantation, as compared to marrow cells which had not been co-cultured. Other investigators have also shown functional effects of microvesicle modulation on target cells. Derugibus and colleagues (121) showed modulation of vascular phenotypes by exposure to endothelial progenitor-derived microvesicles. They demonstrated promotion of endothelial cell survival, proliferation and organization into capillary-like structures *in vitro*. *In vivo*, in severe immunodeficient mice, microvesicle-stimulated endothelial cells organized into patent vessels; this did not happen without microvesicle exposure.

Mechanisms of phenotype change

Initially, we thought that the observed expression of pulmonary epithelial cell-specific mRNA in marrow cells taking up microvesicles was simply due to the transfer of mRNA in microvesicles to the target cells. We had demonstrated pulmonary epithelial cell-specific mRNA inside the microvesicles, showed that microvesicles entered marrow cells and that only the marrow cells which contained microvesicles expressed pulmonary epithelial cell-specific mRNA. However, despite some early results suggesting that RNase exposure of microvesicles inhibited pulmonary epithelial cell-specific mRNA in target marrow cells, more recent work indicated that in most instances, exposure of microvesicles to RNase actually increased expression of pulmonary epithelial cell-specific mRNA in target cells. We found 185 species

of microRNA in these microvesicles with eight having potential lung-specific targets. Thus, these data could be explained by RNase degradation of inhibitory microRNA.

However, we also observed the persistence of pulmonary epithelial cell-specific mRNA expression in marrow cells after three weeks in cytokine-supported culture. This was inconsistent with a simple transfer of mRNA, since we would have expected the RNA to be degraded by this time. We addressed the issue of whether *de novo* transcription was involved in the observed pulmonary epithelial cell-specific mRNA elevations in target marrow cells. Studies with actinomycin D and alpha-amantin, both transcriptional inhibitors, showed predominantly increased expression of the pulmonary epithelial cell-specific mRNA in marrow cells that had been cultured with lung-derived microvesicles, suggesting complex transcriptional regulation (124). In order to address this further, we employed rat/mouse hybrid co-cultures. In these experiments, rat lung was cultured opposite mouse marrow and mouse marrow then evaluated for expression of surfactant C or B mRNA expression. Species-specific primers allowed us to determine whether the observed mRNA was of rat or mouse origin. In every case, the mRNA was of both origins indicating that mRNA was transferred along with transcriptional agents which induced *de novo* surfactant mRNA production in cultured marrow cells. Thus, the mechanisms underlying the genetic phenotype change of target cells is complex, involving transfer of both mRNA and microRNA and of protein-based transcription factors. These phenomena appear to be universal and tissue-specific as we have shown that murine lung, brain, heart and liver tissue will all transfer a tissue-specific phenotype, but not the phenotype of other tissues (124). This concept is shown in Figure 5.

We have presented above a model of stem cell regulation termed “the continuum model” in which the potential of marrow stem cells continually changes with cell cycle transit. We have also shown that the marrow stem cell is a cycling cell. Studies with murine lung-derived microvesicles and murine marrow have now shown that the capacity to take up microvesicles also varies with cycle phase. Thus, phenotype modulation at the stem cell level involves both cell cycle and microvesicle phenotype change. This model is presented in Figure 6.

Thus, one can envision both intra-hematopoietic and extra-hematopoietic cell systems as systems which have a continually changing potential that will only be expressed if there is an appropriate interrogation. In addition, entry of microvesicles into hematopoietic cells varies with cell cycle phase and resets the potentials. One can envision this as represented in a modulogram (Figure 7)

Cancer stem cells and microvesicles

The microvesicle cell modulation also holds for cancer cells. Investigators have shown the movement of cancer phenotype to monocytes (113) and we recently have developed data indicating that both human lung cancer and prostate cancer cells isolated at surgery from patients will move the tissue phenotype to normal human marrow cells (126,127). This opens new strategies for the treatment of cancer. The similarities between cancer cells and normal stem cells also suggest that the concept of a definable cancer stem cell is probably not correct. Rather, there must be numerous cancer cell phenotypes with varying stem cell potential.

Stem Cell Plasticity explained by Microvesicle Cell Phenotype Modulation

We would propose that most of the studies characterizing stem cell or marrow plasticity were in fact explained by microvesicle cell phenotype modulation. This could occur by tissue microvesicles altering the phenotype of marrow or blood cells to the phenotype of the microvesicle originating tissue. Conversely, blood or marrow cells could deliver microvesicles to damaged tissue, restoring the tissue but also delivering the phenotypic markers of the marrow cells. In this latter case, marrow cells would not convert to non-hematopoietic tissue cells but

they would dramatically alter the phenotype of these cells by microvesicle docking, cell entry and genetic modulation. These concepts are presented in Figure 8

We consider this a more satisfactory explanation for the descriptions of stem cell plasticity, which might preferably be referred to as cellular phenotype modulation. One does not have to propose whole cell fusion, dedifferentiation or transdifferentiation to explain the described events with tissue cells showing markers of transplanted marrow.

Conclusions

1. Purification of stem cells is a failed concept; it only contributes information on heterogeneity.
2. Purified stem cells are not representative of marrow stem cells in unseparated marrow populations.
3. The regulation of marrow stem cells is on a cell cycle regulated continuum of potential, which is probably continually altered by exposure to tissue-derived microvesicles. These latter are increased in conditions of injury.
4. The continuum model probably holds for cancer cells along with the concept that there will not be a specific cancer stem cell, but rather a continuously changing population of cancer cells with different potentials.
5. Stem cell plasticity, both intra-hematopoietic and extra-hematopoietic, is mediated by tissue-derived microvesicles acting selectively on cells in different phases of cell cycle. It is a form of mini-multiple cellular fusions through microvesicles.

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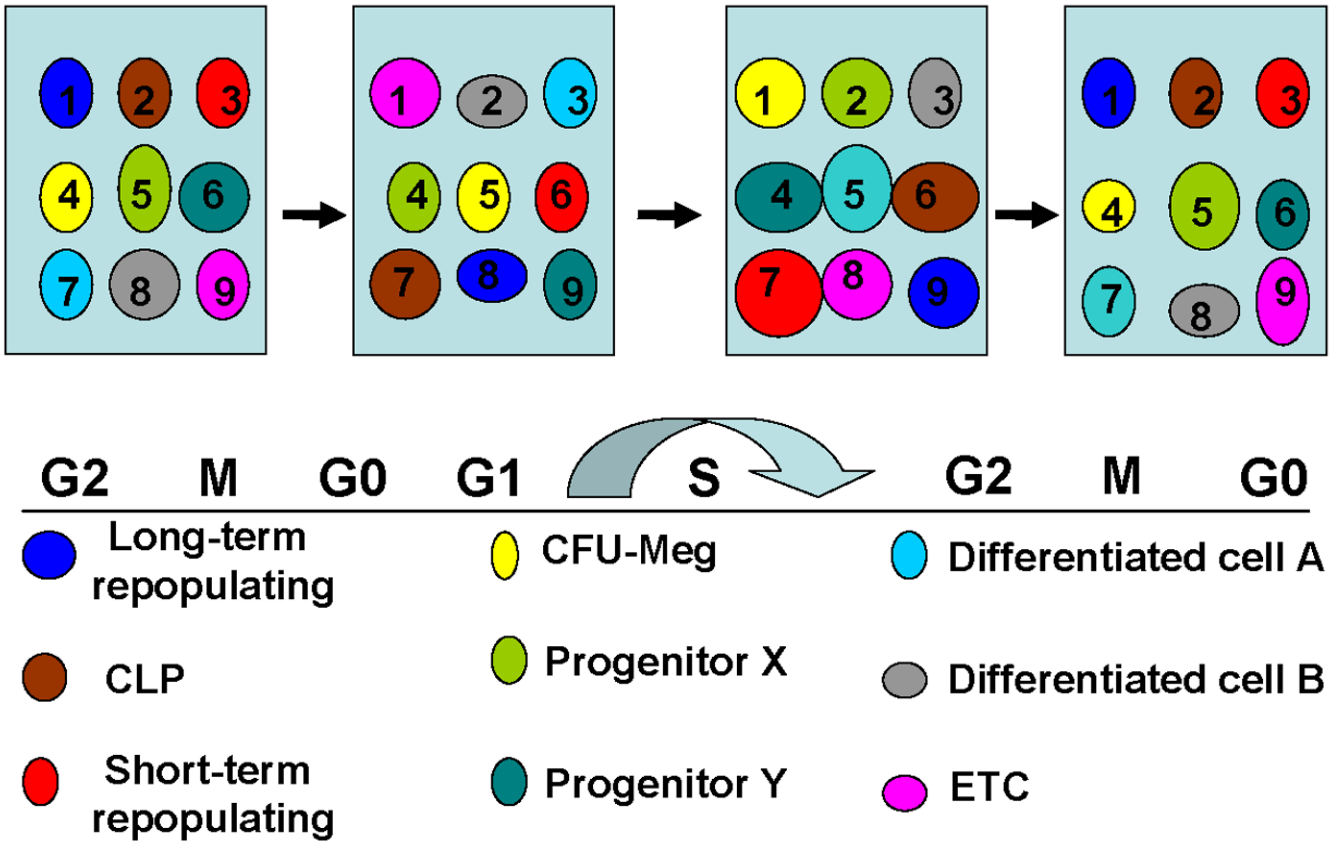

Figure 1. Population model of stem cell phenotype

Numbers and circles represent different phenotypic cell classes. The concept here is that the phenotypes change at different points in cell cycle and eventually return to the original phenotype. For example, cell #1 is a long-term repopulating cell in G2/M/G0 and becomes a different cell in G1, a CFU-Meg in S phase, then returns to the original phenotype. In this model, the individual cell phenotype continuously changes while the population remains stable.

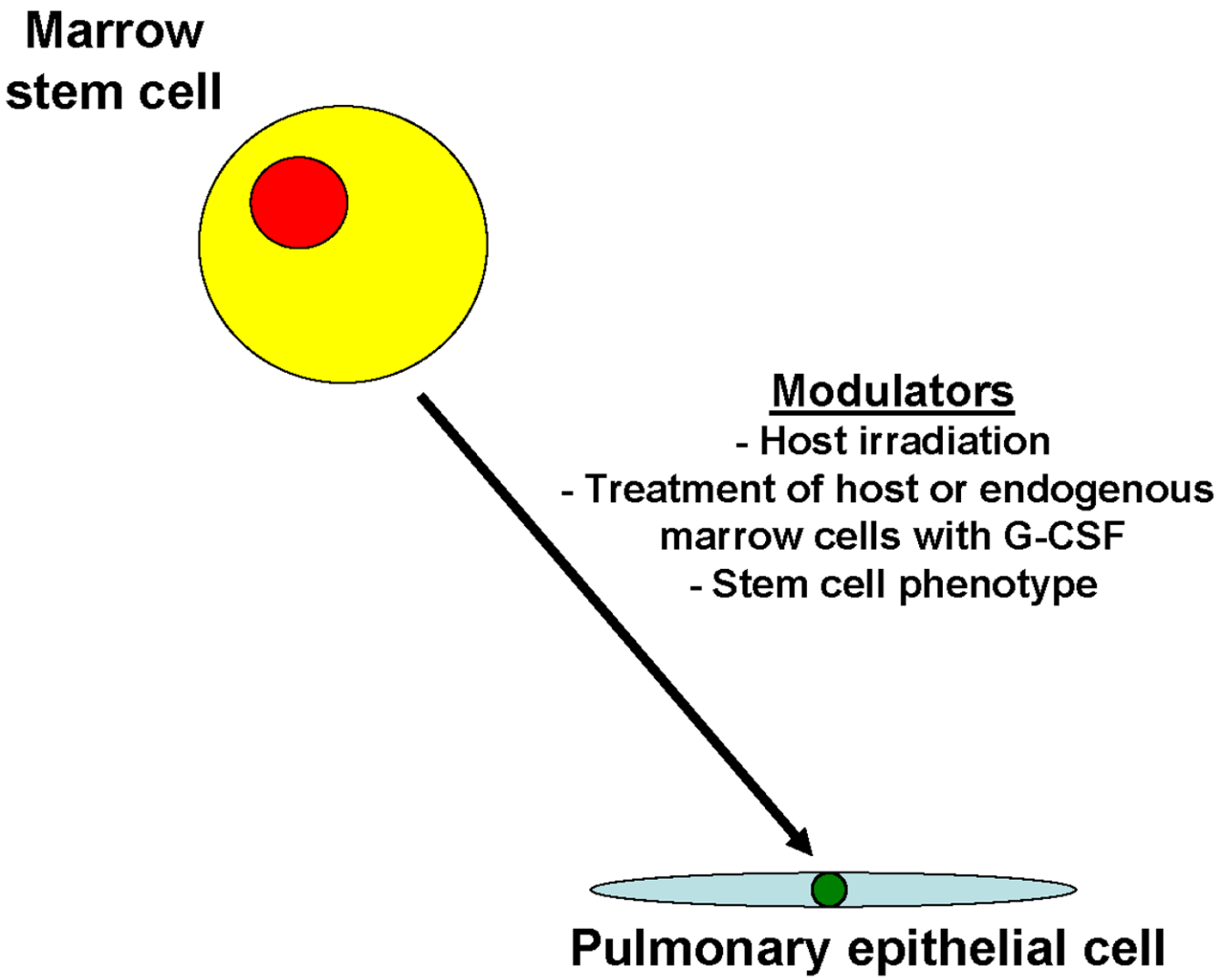

Figure 2. Marrow conversion to epithelial lung cell

This shows conversion of a marrow stem cell phenotype to a pulmonary epithelial cell which is affected by host irradiation, treatment of host or exogenous marrow cells with G-CSF and stem cell phenotype.

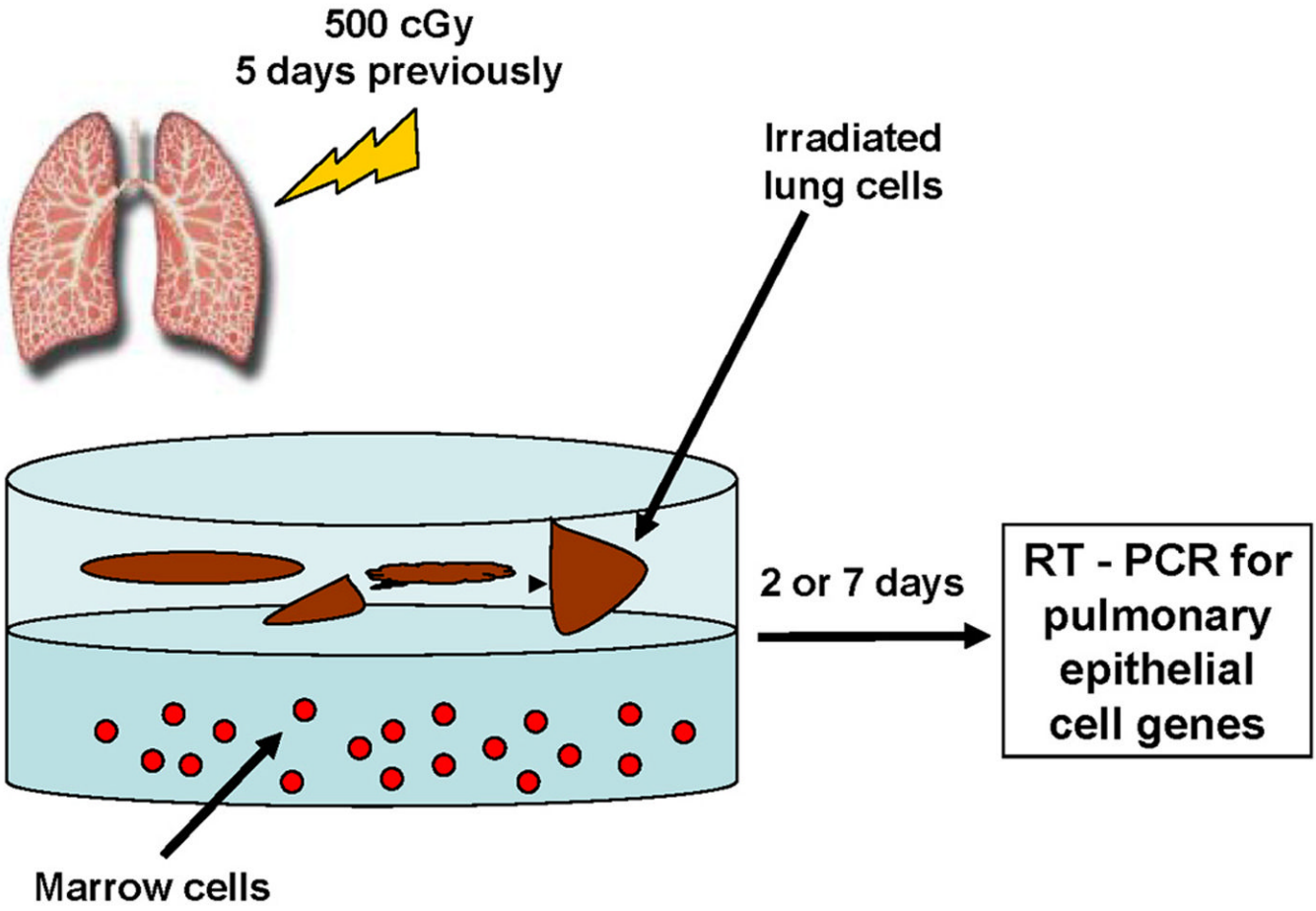

Figure 3. Marrow-lung co-culture

Marrow cells were co-cultured across from lung fragments but separated from them by a cell impermeable (0.4 micron) membrane for two or seven days and expression of pulmonary epithelial genes in marrow cells determined by RT-PCR analysis.

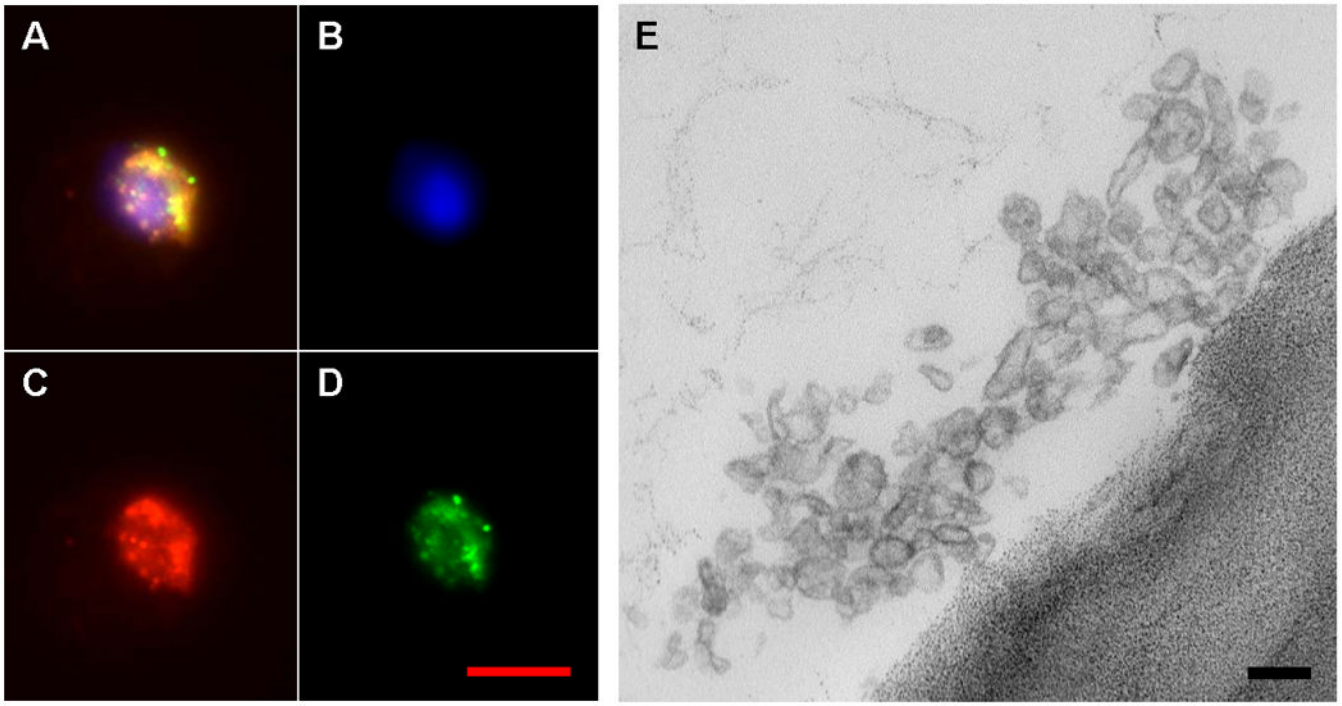

Figure 4. Lung-derived microvesicles

A-D shows a marrow cell with incorporated PKH26 and CFSE-labeled lung-derived microvesicles. A, merged image; B, DAPI filter; C, Texas Red filter; D, FITC filter. Image E is an electron micrograph of FACS-sorted lung-derived microvesicles. Red bar = 10 microns, black bar = 100 nanometers.

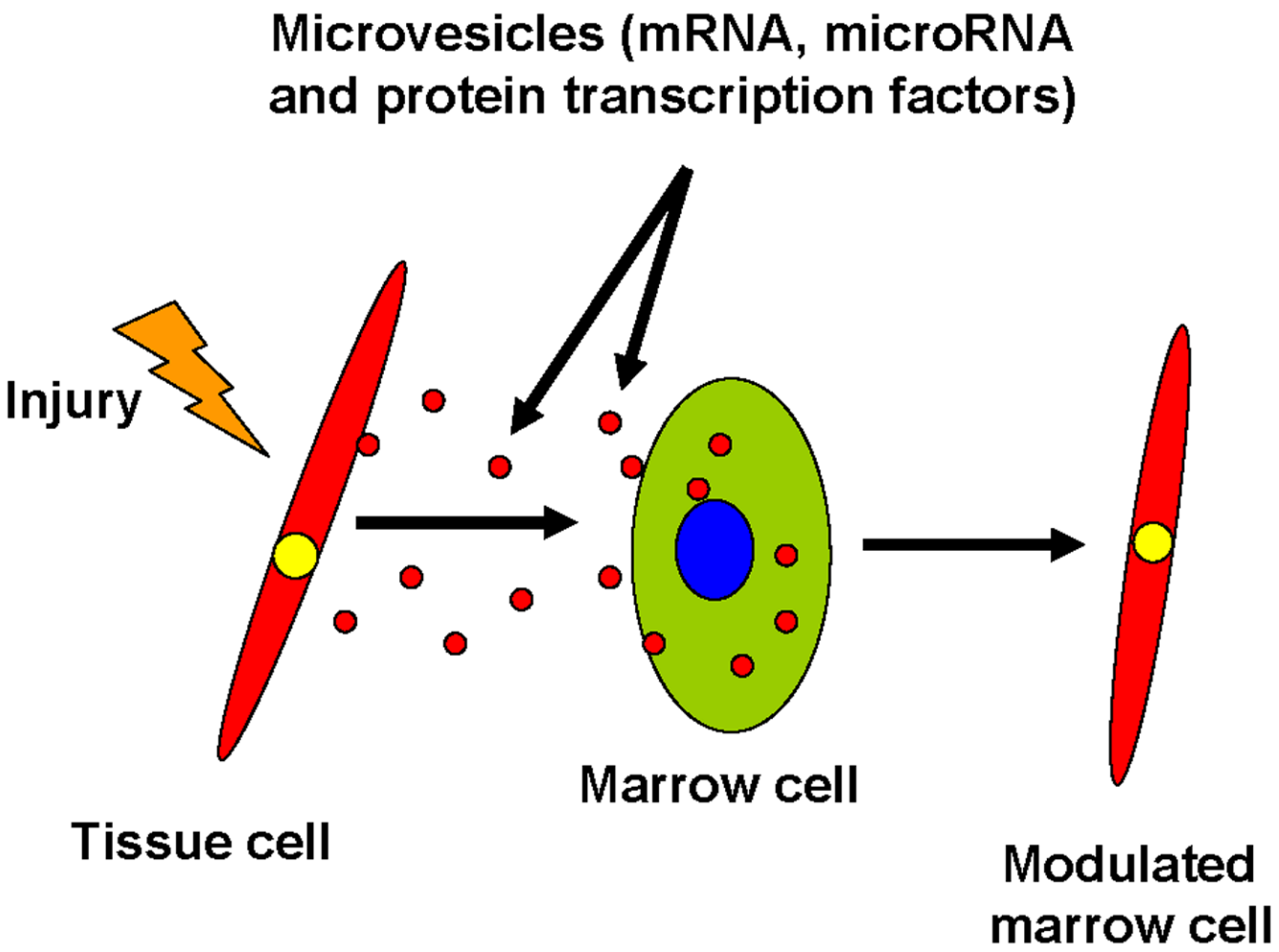

Figure 5. Injury induction of microvesicles

Irradiation injures a non-hematopoietic cell which releases bioactive microvesicles containing protein, mRNA and microRNA. These microvesicles enter marrow cells and alter their phenotype to that of the cell of microvesicle origin.

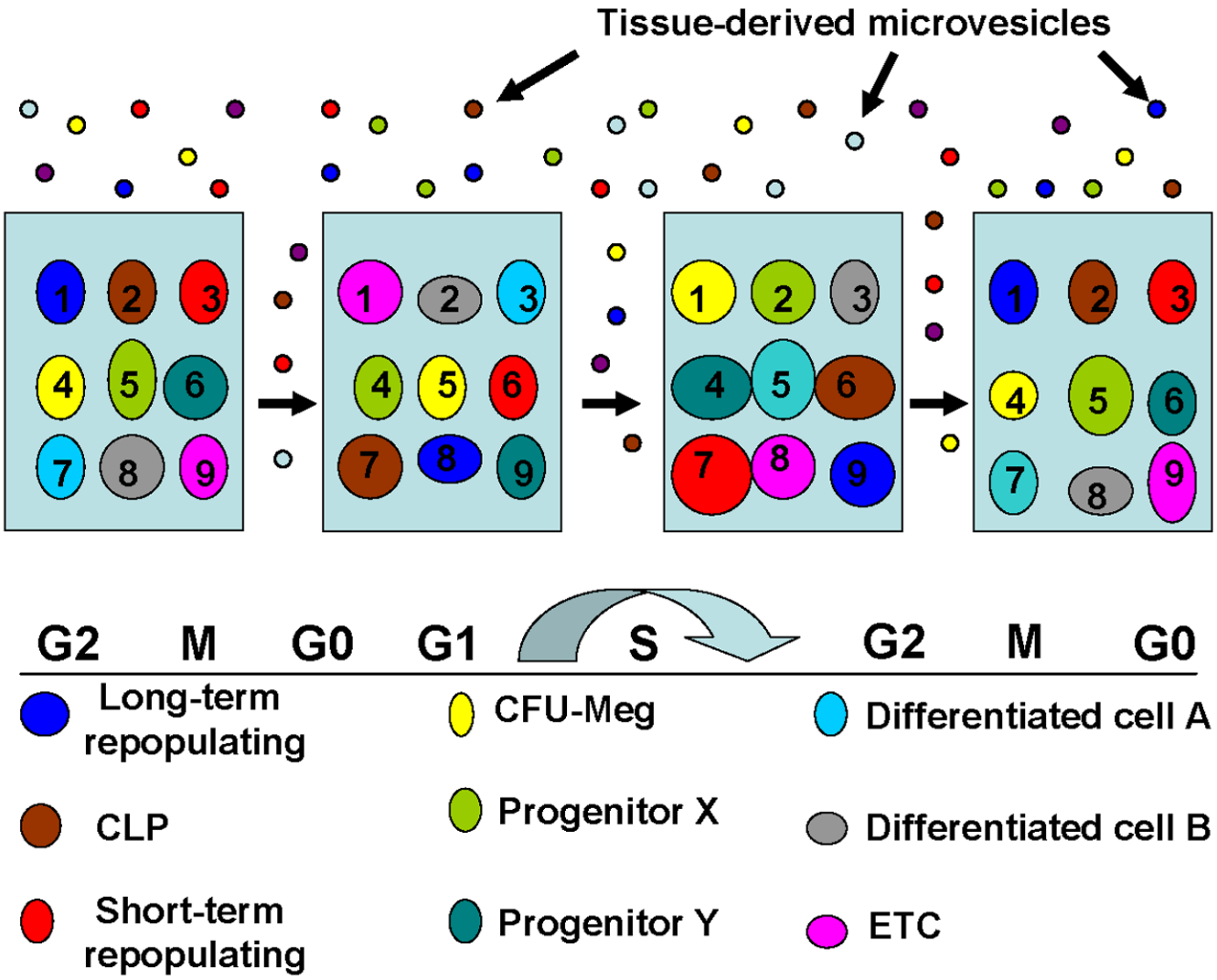

Figure 6. Effect of microvesicles on the stem cell population model

This indicates that microvesicles impose a different order of phenotypic change on stem cells progressing through a cell cycle-related stem cell continuum.

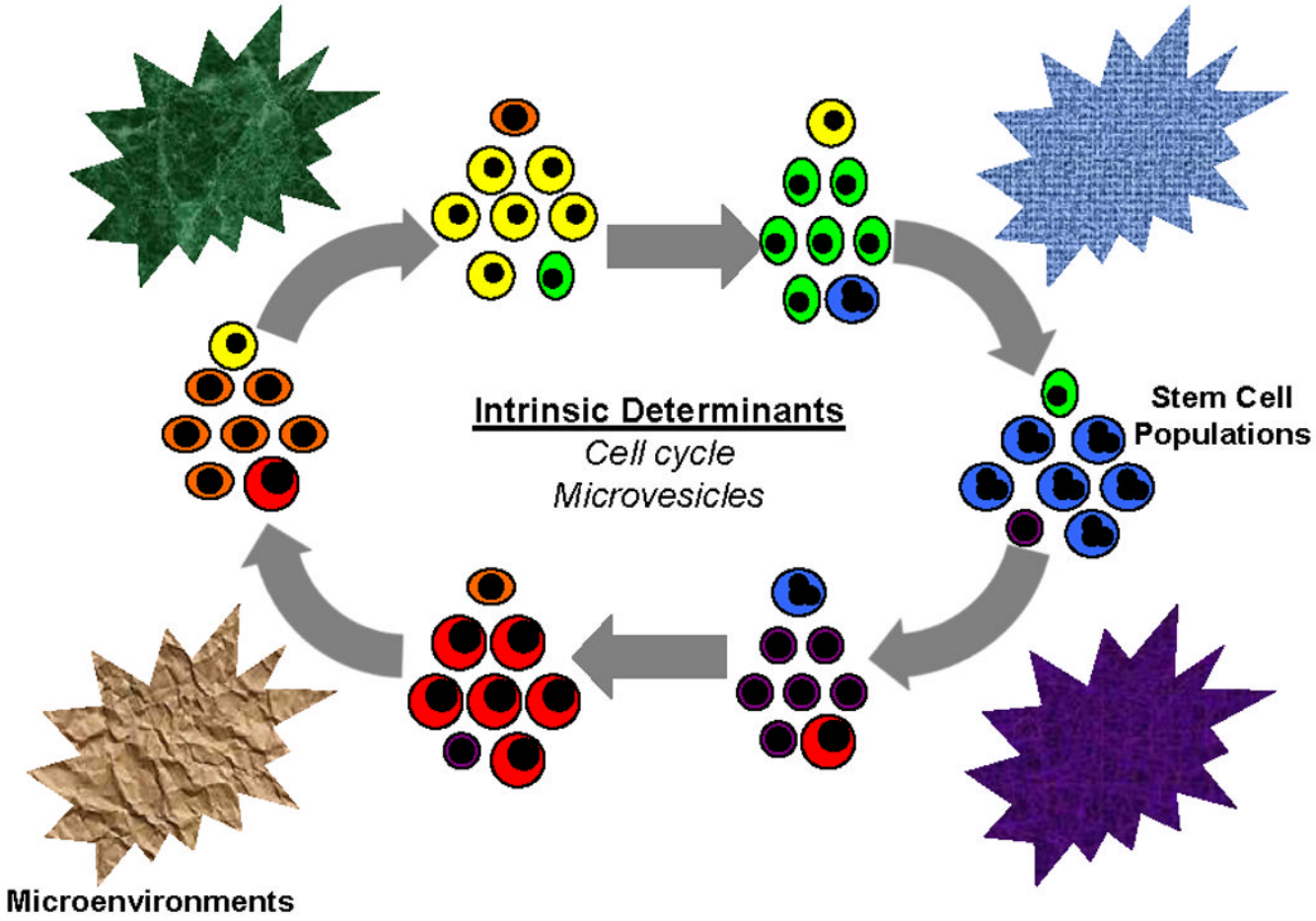

Figure 7. Stem cell modulogram

Stem cells progressing though cycle continuously change individual cell phenotypes while maintaining the population phenotype. This is further modulated by microvesicle cell entry and the final cell fate determined by interactions with different microenvironments.

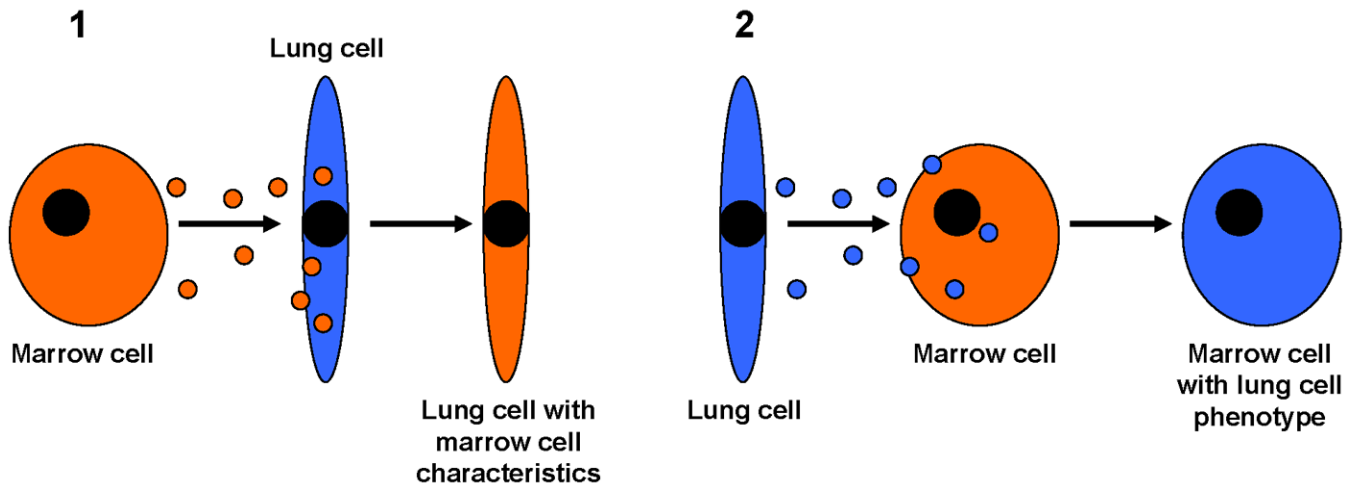

Figure 8. Concepts of stem cell plasticity

Panel 1 indicates that marrow-derived microvesicles may enter lung cells and induce marrow characteristics in the lung cells. Panel 2 indicates than lung-derived microvesicles may enter marrow cells and alter their phenotype towards that of a lung cell.

Table 1

Marrow to Muscle and Lung Conversions

Tissue	Injury	Donor Cells	Conversion Result Tissue Cells (%)	Reference
Skeletal Muscle	Radiation & exercise	GFP- marrow	3.5% (peak)	80
	TBI/mdx Mouse	Spleen & marrow	0.2% (approx)	81
	TBI/mdx Mouse	Marrow side population	1–10%	82
	TBI/cardiotoxin injury anterior tibialis	GFP-marrow Intra-arterial	1–2%	83
	(+direct injection of lineage negative marrow cells)		12.5%	84
	Alpha-Sarcoglycan null dystrophic mice	mesangioblast stem cells	50%	85
Lung	700–950 cGy	GFP marrow, mononuclear cells or side population	1–7% (mixed population, type I pneumocytes)	86
	Non-irradiated	Rosa MAPC	3–5%	87
	(+250 cGy)		(10%)	
	1050 cGy	Fr25/Lin-	20% type II pneumocytes	88
	900 cGy, cardiotoxin or bleomycin lung injury with G-CSF mobilization (x2)	Cytokine treated GFP marrow	35% (peak)	89

TBI: total body irradiation; Mdx: dystrophin deficient mouse

Table 2

Fusion Demonstrated in Converted Cells

Tissue / Cell	Model / Detection	Reference
Hepatocyte	Fah+/+ from Fancc−/− into Fah−/− with NTBC withdrawal. 50% conversion rate. Purified repopulating cells were heterozygous Fah+/+ and Fanc−/−	77
Hepatocyte	Fah+/+ from ROSA26 female marrow into male Fah−/−. Cytogenetic analysis of LacZ+ marrow derived hepatocytes – most with Y chromosome. Karyotypes Fah+/+ 80XXXY or 120 XXXXYY.	78
Purkinje Neuron	GFP to adult mice and both donor and host nuclei found, the Purkinje neurons were stable heterokaryons.	90
Purkinje Neuron, Cardiomyocyte, Hepatocyte	Used Cre/lox recombinase system to show that in marrow transplanted mice all detectable contributions of marrow to nonhematopoietic cell types arose through cell fusion	91
Skeletal Muscle	Murine cardiotoxin injury model male to female, female to male or Rosa B-galactosidase to GFP muscle fibers show both donor and recipient phenotypes. However, mononuclear satellite cells with donor markers suggest conversion to satellite cells occurs without fusion.	83,84

Table 3

Conversions without Fusion

Tissue / Cell	Model / Detection	Reference
Pancreas	Rosa – stop lox and GFP female hosts transplanted with insulin-dependent Cre-male marrow. No GFP+ donor cells in islets.	92
Hepatocyte	Human cord blood to irradiated NOD/SCID mouse. Human hepatocytes with positive protein and chromosome markers, no mouse chromosomes. Conversion rate 1–2%.	93
Hepatocyte	Human cord blood (USSC) into fetal sheep without injury. 20% conversion rate. Microdissected human hepatocytes had only human protein or PCR product.	94
Endothelial	c-kit+, Sca-1+, Lin- into irradiated mouse. Donor endothelia in portal vein. Normal ploidy. Also cord blood to mouse with new blood vessel formation in the eye-no fusion.	95
Renal Mesangial Cells	Male GFP marrow to male mice resulted in numerous GFP+ mesangial cells. None had more than one Y chromosome.	96
Epithelial Cells in Lung, Skin, and Liver	Cre/lox recombinase system. Transplant Z/EG marrow into Cre expressing mice. No mice expressed GFP indicated that fusion had not occurred.	97
Skeletal Muscle	Converted mononucleated satellite cells preceed muscle fiber fusion.	80