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Midbody accumulation in breast cancer stem cells

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14. ABSTRACT

Breast cancer is a complex disease that develops from epithelial lesions confined to breast ducts and lobules and progresses rapidly to become locally invasive and finally metastatic. Our recent studies show that breast cancer cells undergo asymmetric events during cell division that generate different daughter cells. One daughter receives the singular midbody (MB) that is made during every cell division. The cell with this so-called postmitotic *midbody derivative* accumulates additional MBds with successive divisions. In breast tumor sections, rare cells stain for MBds adjacent to the basal layer, the position of putative breast cancer stem cells. MBds are present in high numbers in several human breast cancer cell lines and in human tumors, but are rarely found in normal breast epithelial cell lines or breast tissue. MBds are also found in several well-characterized mouse and human stem cell niches but not in adjacent transit amplifying or differentiating cells. These results suggest that MBds are in almost exclusively in stem cells and putative breast cancer stem cells (CSS). This idea is consistent with the emerging view that breast cancer develops from transformation of stem cells.

Based on these observations, we propose that MBds 1) will serve as markers for breast CSCs, 2) may have diagnostic/prognositc value for breast cancer progression and 3) could directly contribute to breast carcinoma. To test this, we propose the following aims: 1) Quantify MBds in breast tumors and cell lines and compare with normal breast epithelial cells. 2) Test MBd-containing breast cancer cells for CSC activities in vivo and in vitro. 3) Test MBds for their ability to confer breast cancer stem cell properties by disrupting MBd inheritance or RNAi.

15. SUBJECT TERMS

breast cancer, stem cells, midbody, mitosis, microtubules, autophagy, NBR1, Cep55

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INTRODUCTION:

Breast cancer is a complex disease that develops from epithelial lesions confined to breast ducts and lobules and progresses rapidly to become locally invasive and finally metastatic. Our recent studies show that cancer cells undergo a series of asymmetric events during cell division that generate *different* daughter cells (Gromley et al, Cell, 2005). We have shown this is also the case for breast cancer cells (MDA-MB-231, MCF-10A CA1a). Remarkably, one daughter inherits the midbody (MB), an organelle that assembles between the two dividing daughter cells. We call this post-division MB, the *midbody derivative (MBd).* Remarkably, the cell with the MBd accumulates additional MBds with each subsequent cell division. In human breast tumor sections, we observed rare cells adjacent to the basal layer--the position of putative breast cancer stem cells (CSCs)--that stain for MBds. In the breast cancer cell line MDA-MB-231 MBds were present in high numbers. High MBd numbers were also found in several stem cell compartments and in human embryonic stem cells (hESCs, H9, iPS), and they dramatically decreased when stem cells were induced to differentiate. MBds were *not* detected in normal and near normal breast epithelial cells (e.g. HMECs, MCF10A), dividing hepatocytes in regenerating liver, activated T cells or transit amplifying cells in all tissues studied. These results suggest that MBds are present in normal SCs and breast CSCs in vivo and in vitro. Our work is consistent with the emerging view that breast cancer develops from transformation of stem cells (Dontu et al, 2005; Ponti et al, 2005). We propose that MBds 1) will serve as markers for breast CSCs, 2) may have diagnostic/prognostic value for breast cancer progression and 3) could directly contribute to breast carcinoma. To test this, we propose the following aims: 1) Quantify MBds in breast tumors and cell lines and compare them with normal breast epithelial cells. 2) Test MBd-containing breast cancer cells for CSC properties in vivo and in vitro. 3) Test MBds for the ability to confer breast cancer stem cell properties by disrupting MBd inheritance or by RNAi.

BODY: We have made key discoveries in MBd function in cancer and stem cells in this funding period. These are described below.

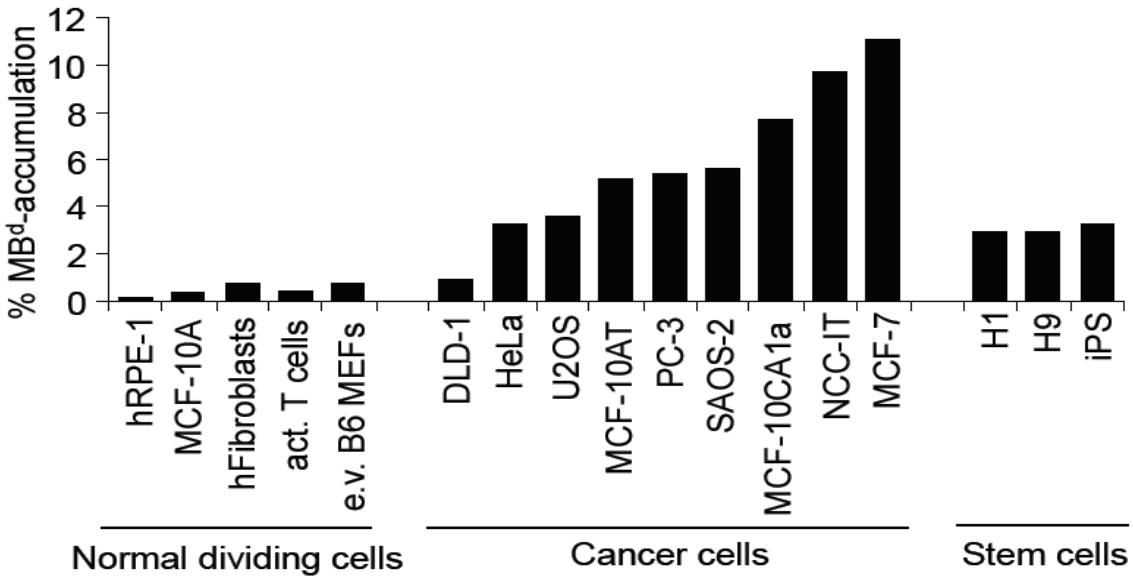

Figure 1. Breast cancer cells accumulate midbody derivatives

MBd accumulation is enhanced in cells derived from breast cancers and other cancers.

To gain insight into cell type differences in MBd accumulation, we compared MBd levels in cancer cells, stem cells, and normal dividing cells (Fig. 1). In general, primary and telomerase-immortalized cells had very low MBd accumulation levels (Fig. 1, left). Several hESCs and iPS cell lines showed significantly higher MBd accumulation levels (~7-fold, Fig. 1, right). With one exception, cells derived from a diversity of human cancers showed the highest level of MBd accumulation (Fig. 1, center). Of note, was the high level of MBd accumulation in the breast cancer cell lines MCF-10AT and MCF-10CA1a, compared with their non-tumorigenic parental line, MCF-10A, suggesting a possible relationship between MBd accumulation and tumorigenicity. The highest level of MBds was observed in the breast cancer line MCF7. The significance of this is currently under investigation. Some light is shed upon this in figures that are included in the figures below. The common ability of cancer cells and stem cells to accumulate MBds suggests a relationship between MBd-accumulation and potential cancer 'initiating' or 'stem' cells defined by the cancer stem cell theory. This idea has been explored in more detail in the work outlined below. In addition, studies are underway to test for the presence of MBds in breast cancer tissues. Preliminary data demonstrates that MBds appear to be elevated in breast tumor tissue compared with the surrounding normal tissue.

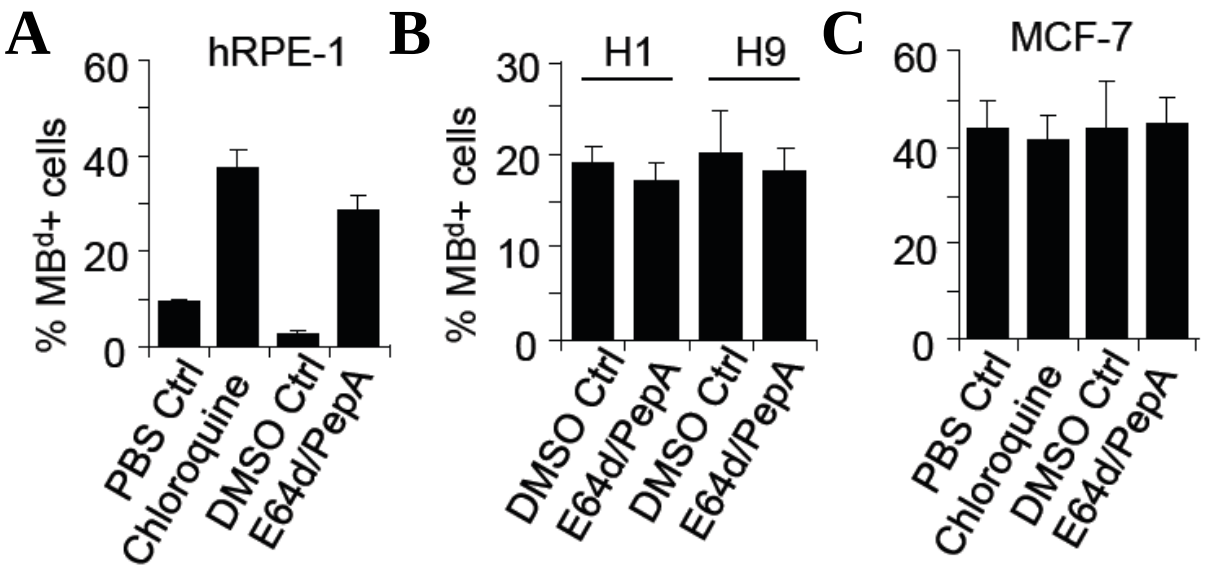

Figure 2. A-C. Lysosome inhibition (chloroquine, E64d/PepA) increases MBds in RPE-1 cells **(A),** but not in stem cells **(B,** H1, H9) or cancer cells **(C,** MCF-7). **D.** NBR1 depletion by shRNA treatment elevated MBd levels in human fibroblasts compared to controls (shNT).

Stem cells and cancer cells evade lysosomal degradation of MBds

To understand why different cell types accumulate MBds to different levels, we performed a series of tests for the loss and retention of MBds by cells. We found that MBds were often found within lysosomal membrane boundaries in MBd-poor normal differentiating cells and MBds could accumulate in these cells upon lysosomal inhibition with chloroquine to neutralize lysosome pH or with peptide inhibitors of a subset of lysosomal enzymes (E64d/PepA) (Fig. 2A above). These results are consistent with lysosomal degradation of MBds in normal cells. Because MBds accumulate in breast cancer cells, other cancer cells and stem cells, we reasoned that these cells might evade lysosomal degradation. In fact, lysosomal inhibition in MBd-rich cells (hESC, MCF-7) did not affect the level of MBd+ cells or MBds within lysosomes (Fig. 2B, C), despite the fact that the degree of lysosomal inhibition was similar in normal, cancer and stem cells (not shown). We propose that MBd-accumulating cells (stem cells, cancer cells) have a low capacity to deliver MBds to lysosomes, so MBds are retained in the cytoplasm, avoid lysosomal degradation and accumulate. MBd-poor cells deliver MBds to lysosomes where they are degraded, preventing accumulation. However, the mechanism for MBd delivery to lysosomes was not identified in these analyses.

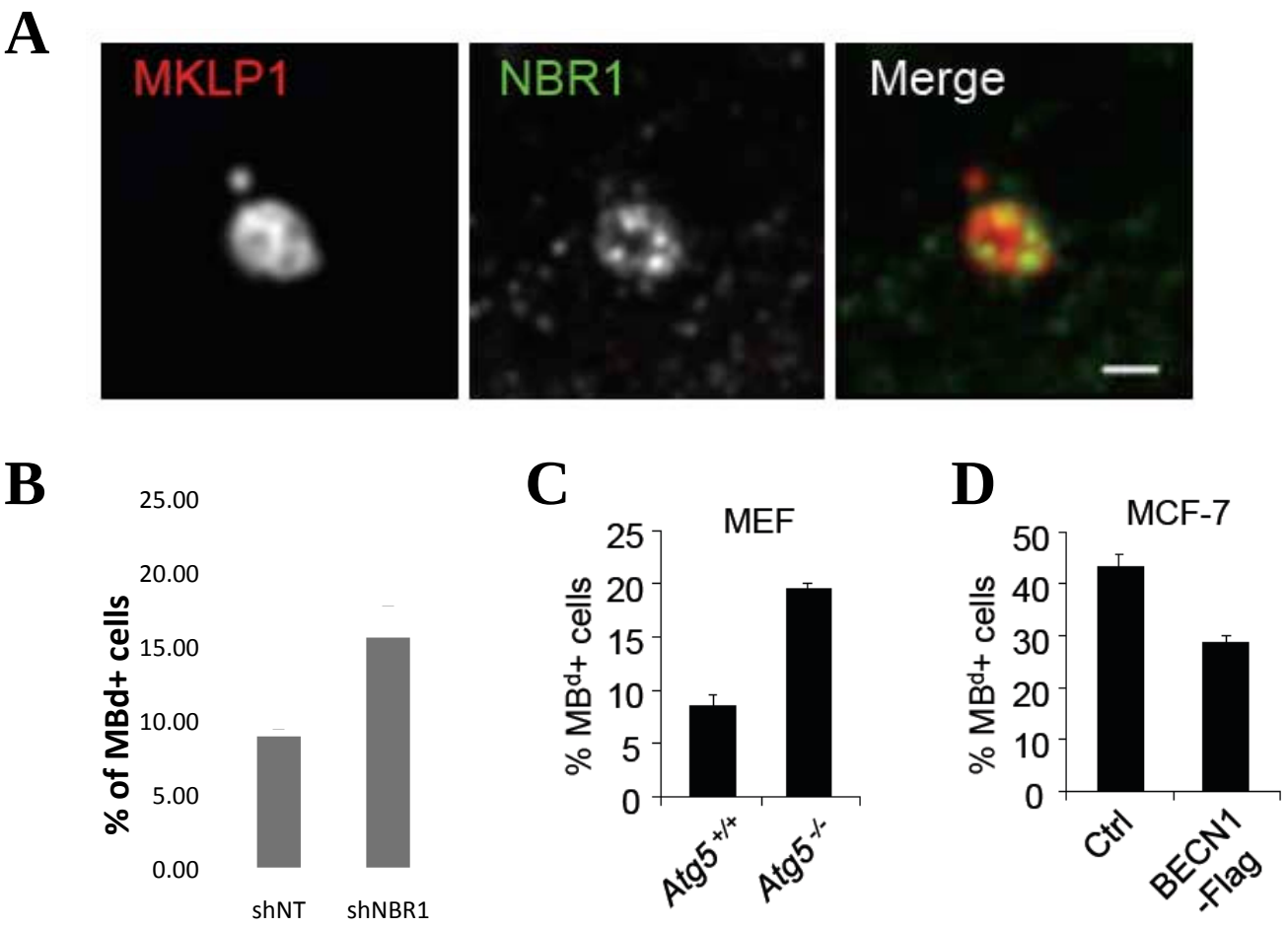

Figure 3. MBds are degraded by NBR1-mediated autophagy. **A.** NBR1 localizes to MBds. **B.** NBR1 depletion by shRNA in MCF7 cells increases MBd numbers (shNBR1**)** over control (shNR). **C.** Inhibition of autophagy in MEFs from the Atg5 knockout mouse (Atg5-/-) causes an increase in the % of MBd+ cells compared to wild type (Atg5+/+). **D.** Activation of autophagy in MCF-7 breast cancer cells (BECN1-Flag) causes MBd loss.

Receptor-mediated autophagy is involved in MBd degradation

To address the question of how MBds are targeted to the lysosomal pathway, we examined degradation pathways in cells. We identified the pathway that eliminates organelles and cytoplasmic constituents, autophagy, as a major contributor to MBd degradation. We identified NBR1 as an autophagic receptor for MBd degradation in this pathway. NBR1 localized to MBds (Fig. 3A) and its depletion caused MBd accumulation (Fig. 3B). We obtained cells deficient in autophagy, namely MEFs from mice lacking autophagy gene Atg5. In these cells, MBd levels were elevated compared to control MEFs (Fig. 3C). In the breast cancer cell line, MCF-7, we observed a decrease in MBds when autophagy was increased by beclin expression (Fig. 3D, BECN1-Flag).

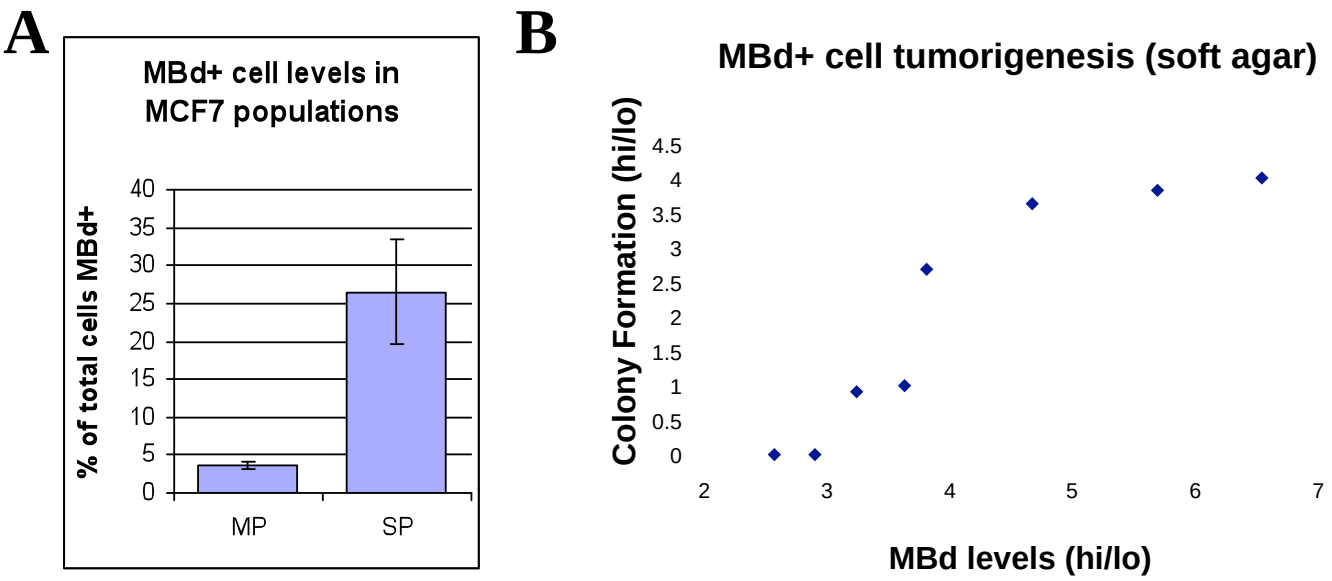

Figure 4. Breast cancer cells with MBds have enhanced tumor-like properties. **A.** MBds are found in the cancer cell side population (SP) compared to the main population (MP). **B.** MBd+ cells show increased growth in soft agar (up to 4-fold higher)

In vitro tumorigenicity is enhanced in MBd accumulating cancer cells

Because MBds selectively accumulate in stem cell niches, hESCs and iPSCs, we hypothesized that they may also accumulate in a subpopulation of cells thought to have cancer-initiating properties, the cancer stem cells (CSCs). In fact, MCF-7 CSCs derived from the main population based on their ability to efflux Hoescht 33342 dye (the side-population or SP) showed a 7-fold increase in MBd+ cells over the main population (MP) (Fig. 4A).

To *more directly* address the role of MBds, we used fluorescence activated cell sorting (FACS) and cell lines expressing GFP-MKLP, a component of the MBd. These strategies were exploited to isolate populations of HeLa cells with high and low percentages of MBd+ cells (MBd high and MBd low, respectively). These different cell populations were subsequently tested for anchorage-independent growth in soft agar. A strong trend was observed between increased colony formation and increased percentage of MBd+ cells (Fig. 4B) showing the highest number of soft agar colonies in preparations where the separation of MBd+ cells was highest. In another assay, MBds were enriched by depleting the receptor for autophagic degradation of MBd (NBR1) and increased colony formation was observed (2-fold increase). Both results suggest that MBds contribute to tumor phenotypes of breast cancer stem cells.

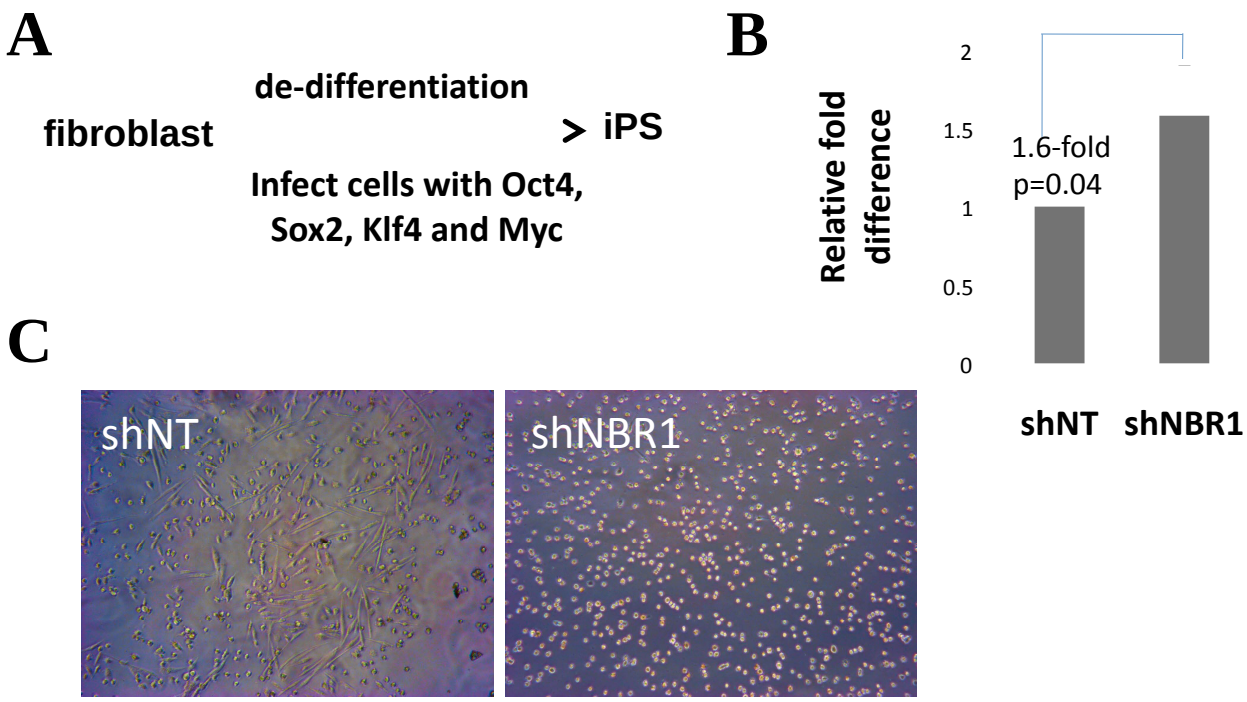

Figure 5. A. Scheme for fibroblast reprogramming into pluripotent stem cells (iPS). **B.** Depletion of the autophagic receptor for MBds (**B,** NBR1) decreases the level of MBd+ cells (Fig. 3) and increases the number of stem cell colonies (**B). C.** Myotubes form in control (left) but not in NBR1 depletion (right) showing retention of stem cell characteristics in MBd-high NBR1 depleted cells.

Reprogramming efficiency is enhanced following MB$^{\alpha}$-degradation

We next tested the role of MB^{d}s during reprogramming of fibroblasts to induced pluripotent (iPS) cells (Fig.5A). Fibroblast (dH1f) cell lines stably expressing NBR1-specific shRNAs (NBR1) or control shRNAs (NT) were constructed. As expected, more NBR1 cells accumulated MBds than NT cells (Fig. 3) and NBR1-silencing did not affect autophagic activity (data not shown). After reprogramming, the number of iPS colonies positive for the stem cell marker tra-1-60+ was significantly greater in NBR1 cells (~1.6 fold > NT cells, Fig. 5B) than controls. This demonstrates that reprogramming is more efficient after MBd enrichment mediated by NBR1-silencing. When adult muscle stem cells were differentiated in cells depleted of NBR1, differentiation was inhibited (myotubes were not formed and stem cell activity was retained) at early times after induction of differentiation (Fig. 5C). Taken together, data from three independent strategies suggests that MBds may be involved in the maintenance of breast cancer stem cells, other cancer cells and stem cells. ***We believe that the use of normal, cancer and stem cells provides a powerful approach to further characterize the properties of breast cancer 'stem cells'.***

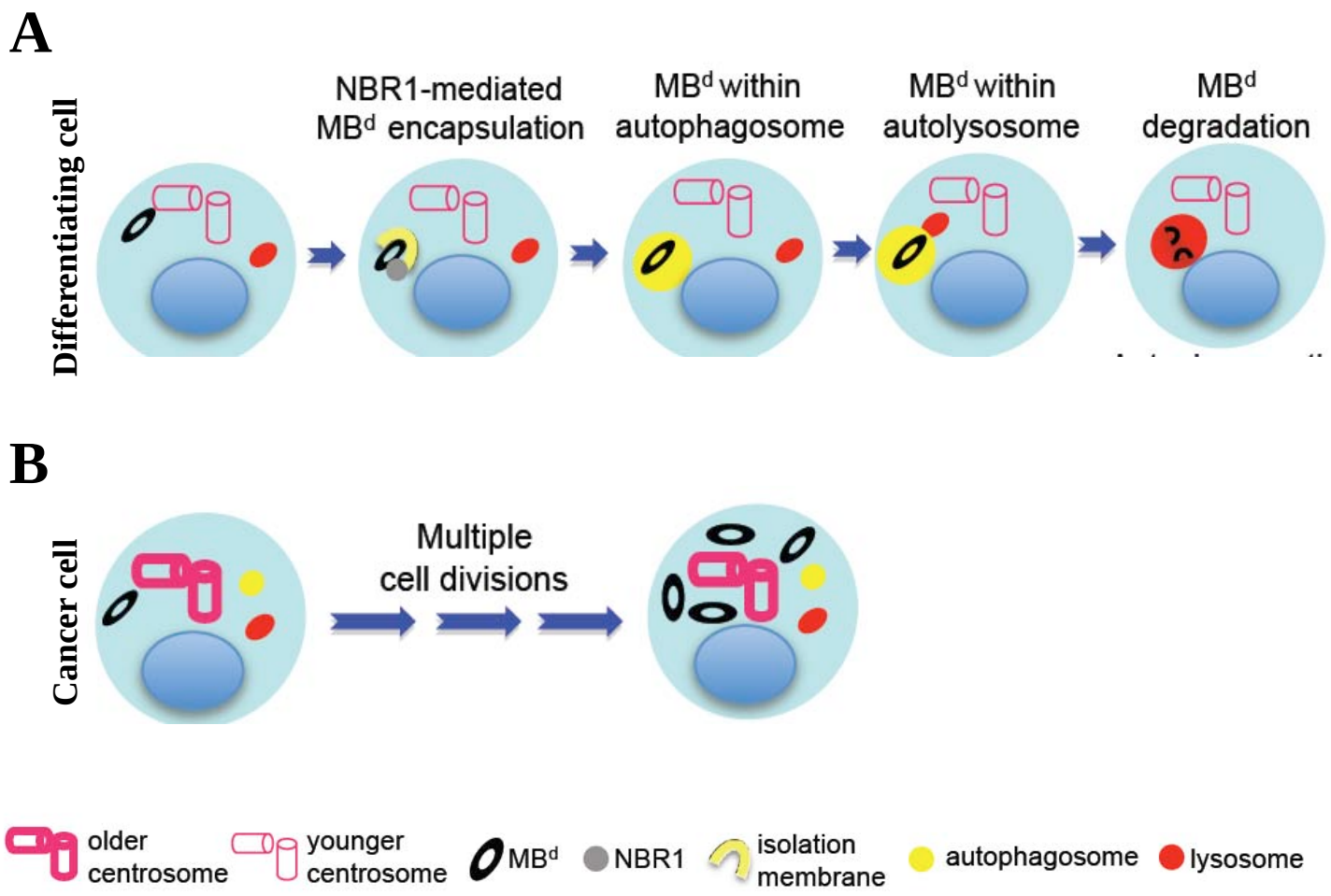

Figure 6. Model for MBd fate in cancer cells and normal cells. A. In normal differentiating cells, NBR1 mediates encapsulation of MBds by autophagosomes, which fuse with lysosomes to degrade MBds. **B.** In cancer cells and stem cells, MBds are not degraded and accumulate.

KEY RESEARCH ACCOMPLISHMENTS:

*Our work during the last funding period (below) suggests that MBds contribute to the function of stem cells and cancer stem cells.
*We identified an autophagic protein (NBR1) that targets MBds to the autophagic pathway in breast epithelial cells but not breast cancer cells.
*Normal breast epithelial cells (MCF10A) lose MBds by release from cells in addition to autophagy
*MBds in breast cancer cells evade NBR1-specific degradation and release from cells.
*The MBd protein, Cep55, binds NBR1 and targets MBds to the autophagic pathway in breast epithelial cells but not breast cancer cells.
*MBd-positive MCF7cells are enriched in cancer cell side populations (SP).
*MBds in human fibroblasts accumulate when NBR1 is depleted by siRNA and they form more stem cell colonies when reprogrammed to form induced pluripotent cells (iPS).

REPORTABLE OUTCOMES:

* Manuscript: Tse-Chun Kuo, Chun-Ting Chen, Desiree Baron, Cara Weismann, Jean-Marie Houghton and S
Doxsey. Midbodies accumulate in stem cells through asymmetric inheritance and evasion of NBR1-mediated autophagy. Nature Cell Biology, in revision.
*Abstract: Tse-Chun Kuo, Chun-Ting Chen, Desiree Barron, Stephen Doxsey. Selective accumulation of midbodies in stem cells. American Society of Cell Biology, Dec. 5-9, 2009, San Diego, CA 2009.
* Based in part on the work in this proposal, I continue to serve as Chair of the search committee for a Director of UMass Medical School's Stem Cell and Regenerative Medicine Institute.
* Based on work in this proposal, I received several invitations to give seminars:
2009-2010:
07/2009 Little People of America Meeting, Brooklyn, NYC
10/2009 Memorial Sloan-Kettering Cancer Center, NYC
11/2009 Worcester Polytechnic Institute, Worcester, MA 2010
01/2010 ASCB Course, Dar Es Saalam, Tanzania, Emerging Roles of Centrosomes
03/2010 Keystone Symposium, Monterey, CA, Cilia protein function in mitosis and aneuploidy
05/2010 Polycystic Kidney Disease and cancer, Harvard, Boston, MA
08/2010 UMass Amherst, Microtubule meeting, Amherst, MA
08/2010 Woods Hole, MA, Ellison Medical Foundation Annual Meeting
09/2010 Cell Cycle, Mitosis and Cancer, Roscoff, France
10/2010 Cell cycle and cancer meeting, Loutraki, Greece
11/2010 Plenary Lecture, Cell Cycle and Cancer meeting, Sydney, Australia

CONCLUSION: We have made progress on the aims of our proposal and made important progress into the role of MBds in cancer-like properties of breast cancer cells. We have shown that MBds are not degraded or released from cells in breast cancer cells as they in normal breast epithelial cells. Flow cytometric separation of MBd+ and MBd- cells showed that MBd+ cells form more and larger colonies in soft agar and are found primarily in cancer 'stem cell' side populations. These restuls indicate that the MBd+ cells have stem cell like properties. Consistent with this is the observation that stem cells induced to form from fibroblasts after reprogramming show greater numbers when extra MBds are present. Morover, we identified a new pathway for autophagic degradation that involves the autophagic protein NBR1 coupled to the MBd protein Cep55. This protein pair directs MBds to the autophagy pathway for degradation in breast epithelial cells. In breast cancer cells, this pathway if evaded. We also found that MBds can be lost from cells by being jettisoned from the cell into the media or extracellular space. These results have important implications for targeting MBds for therapeutic purposes.

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A. Gromley et al, Cell 123, 75-87, 2005.
D. Ponti et al, Cancer Res. 5506-11, 2005.
G. Dontu et al, Stem Cell Rev, 207-13, 2005.