

Neosis - A Parasexual Somatic Reduction Division in Cancer

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ABSTRACT We have recently reported a novel type of cell division involved in the origin and growth of cancers. Termed neosis, as opposed to mitosis and meiosis, this type of cell division occurs only in senescent polyploid giant cells and not in normal diploid cells. Up to ~10% of tumor cells *in vitro* and *in vivo*, are polyploid giant cells and so far there is no explanation for their role in cancer. These resemble senescent cells, which are thought to play a tumor suppressor role. We have shown that such cells have the potential to undergo neosis, a parasexual, somatic reduction division characterized by karyokinesis via nuclear budding, followed by asymmetric cytokinesis, (often) giving rise to aneuploid daughter cells termed Raju cells, which are the progenitors of tumor cells. These Tumor Initiating Raju Cells (TIRCs) are unique in that they transiently display stem cell properties, have inherited genomic instability, differentiate into tumor cells and have extended, but, limited mitotic life span (*MLS). At the end of their extended MLS (EMLS), the tumor cells repeat the cycle of senescence, neosis and production of Tumor Rejuvenating Raju Cells (TRRCs), which repeat the same cycle of events several times through the life of tumor in a progressively non-synchronous fashion. When tumor cells are subjected to chemotherapy or radiotherapy, they undergo premature senescence; but, some cells escape senescence via S/T-neosis and yield TRRCs, whose mitotic progenies may be resistant to genotoxins. Although neosis-like events have appeared in the literature sporadically for more than a century under different names, they were neglected since the significance of such events was not known till now. The data on neosis questions the basic tenets of the current concepts of cancer, i.e., (1) Cancer arises via mitosis, (2) Cancer cells are immortal and (3) Cancer cell continuity is due to the unlimited asymmetric mitotic potential of mutant stem cells or Cancer Stem Cells (CSCs). Neosis paradigm supports the concept that (1) Cancer arises via neosis, (2) cancer cells are not immortal, but undergo repeated senescent phases and that (3) tumor cell lineage continuity is due to escape from senescent phase via neosis, since tumor cells carry mutant or epimutant genes in the senescent checkpoint pathway. Thus, genesis and regeneration of Raju cells via repetitive neotic divisions is responsible for the origin and continuous growth of different tumor types. This concept accommodates epigenetic expression of telomerase, meiotic genes, multidrug resistance genes and stem cell-specific genes in tumor cells and also explains the role of senescent cells found in tumor tissues. Thus, neosis appears to involve global epigenetic modulation (EM), in order to fine-tune the chromatin with DNA damage important for producing reproductively viable genomes from the non-viable polyploid genome, before being discarded by post-neotic death of neosis mother cells (NMCs).

INTRODUCTION

Mitosis and meiosis are the two classical modes of cell division. While mitosis is involved in the division of one diploid mother cell giving rise to two identical daughter cells that resembles the mother cell, meiosis or reduction division results in haploid cells with new combinations of alleles due to the cross-over phenomenon (Kliensmith and Kish 1995). Mitotic division is responsible for the growth and maintenance of the bulk of the body of the multicellular organism from the single celled zygote. Therefore, almost

all of the studies on cell cycle control so far have been focused on understanding the regulation of the mitotic cell cycle. This is evident in our understanding of various diseases including cancer. More than a century of research efforts have increased our knowledge about the molecular events that drive the mitotic cell cycle. These include the various cell cycle phases, and the regulatory signals that govern progressive and sequential events that occur during cell division, DNA damage response, apoptosis, the mitotic life span (*MLS) of normal diploid cells, the on-set of senescent phase at the end of the MLS, immortalization and neoplastic transformation (Sundaram et al. 2004).

Primary diploid human cells have a finite MLS, after which they stop dividing, become senescent and eventually die (Hayflick and Moorehead 1965). Apoptosis or programmed cell death has

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been recognized as a major deterrent for the growth of cancer for several decades now (Kerr et al. 1972). However, the phenomenon of senescence as a tumor suppressor mechanism both *in vitro* and *in vivo* has been confirmed only recently (Reviewed in Campisi 2005; Braig and Schmitt 2006; Rajaraman et al. 2006). Senescence is characterized by large, flat cell morphology, and is often accompanied by nuclear abnormalities, including polyploidy, and multinucleation. They positively stain for senescence-associated β -galactosidase at pH 6.0 (SA- β -gal) and senescence-associated heterochromatin formation (SAHF) (Dimri 2005). Further, senescence is accompanied by shutting down of mitotic genes and activation of anti-mitotic genes (Roninson et al. 2001; Dimri 2005). Senescence can be induced by different mechanisms, which fall into two groups: (1) replicative age-induced senescence in normal diploid cells due to telomere attrition and (2) genetic stress-induced premature senescence in both normal and tumor cells after exposure to genotoxins or by inadvertent activation of an oncogene. These senescence signal transduction pathways appear to involve the p53/RB/p16INK4a signaling network of tumor suppressor genes (Shelton et al. 1999; Sherr and DePinho 2000; Sharpless 2004; Roberson et al. 2005) and either mutation or epimutation of any of these genes is sufficient to bypass senescence and to initiate tumor growth by an unknown mechanism. Such cells are thought to be 'immortal' and have gained unlimited division potential (Wright and Shay 1992; Shelton et al. 1999; Reviewed in Rajaraman et al. 2005, 2006).

What is Neosis?

We recently reported a novel mode of cell division termed neosis, which is involved in bypassing senescence and therefore, is responsible for the initiation and continuous growth of different types of tumors. Neosis occurs only in senescent, polyploid cells, and never occurs in normal diploid cells. Neosis is a parasexual, somatic, reduction division displayed by a subset of senescent, multinucleate and / or polyploid giant cells (MN/PGs) formed during the spontaneous senescent phase of normal cells at the end of their MLS or genetic stress-induced accelerated senescence phase in normal and tumor cells (Sundaram et al. 2004; Rajaraman et

al. 2005, 2006). It is characterized by (1) chromosome distribution to daughter cells via nuclear budding in the presence of an intact nuclear envelope, (2) followed by asymmetric cytokinesis, giving rise to an indefinite number of small, aneuploid, mitotically active cells termed Raju cells (Raju meaning King in Telugu language), after which the polyploid neosis mother cell (NMC) dies. Raju cells display transient stem cell-like properties, and mature into tumor cells with extended, but, limited mitotic life span (EMLS), finally arriving at a secondary/tertiary senescent phase. Neosis is interspersed with the EMLS of Raju cells and their senescent phase and is repeated several times during tumor growth in a progressively non-synchronous fashion. When a cell undergoes neosis for the first time, this produces the Tumor Initiating Raju Cells (or TIRC) and this is termed the primary neosis or P-neosis. When a cell undergoes more than one neotic division, the subsequent neotic divisions are termed Secondary or Tertiary neosis or S/T-neosis, which yield Tumor Rejuvenating Raju Cells or TRRCs. Further, neosis is also responsible for the outgrowth of resistant tumor cells after exposure to genotoxins. Thus, neosis appears to be the mode of origin and continuous growth of different tumor types, including hematological malignancies, carcinoma and sarcomas (Sundaram et al. 2004; Rajaraman et al. 2005, 2006).

Neosis Gives Rise to Cancer Cells

Transformed focus formation assay is the *in vitro* equivalent of *in vivo* tumorigenesis (Reznikof et al. 1973; Heidelberger et al. 1983). We started studying the process of transformed focus formation at the single cell level in 1998, using computerized video time-lapse microscopy after exposing C3H10T1/2 cells to genotoxins such as etoposide or radiation. Our studies revealed that transformed focus formation occurred only in a subset of premature senescent cells induced by genotoxins and this occurred by karyokinesis via nuclear budding in the presence of an intact nuclear envelope, followed by asymmetric cytokinesis and cellularization yielding an indefinite number of daughter cells. Unlike in the cases of mitosis and meiosis, where the nuclear membrane is disassembled during karyokinesis, a process known to activate spindle checkpoint or mitotic checkpoint (Kops

et al. 2005), during neosis, the nuclear envelope is kept intact during genome distribution to the newly formed nuclear bud. We termed this mode of cell division neosis, the daughter cells the Raju cells, and the senescent MN/PGs the neosis mother cells (NMCs). Raju cells by definition means the nascent neotic daughter cells, which have not undergone mitotic division yet (Rajaraman et al. 2005), since their surface characteristics may change after they undergo mitotic division (Rajaraman et al. 2006; see below). The NMCs died immediately after yielding about 10-12 Raju cells, and the latter displayed genomic instability, aneuploidy, and extended mitotic life span (EMLS), probably by reactivation of telomerase (Kim et al. 1994; Counter et al. 1994, 1998; Meyerson et al. 1998) and matured into tumor cells.

Similarly, spontaneous transformation of p53^{-/-} mouse cells also occurred only at the end of MLS of diploid cells and was preceded by spontaneous senescent phase and neosis. In p53^{-/-}MGB.MEF cells, 100 % of cells turned MN/PGs and each cell produced about 50 or more TIRCs spontaneously. Within about 30 days after P-neosis, the Petri plate was full of spontaneously transformed cells and no NMCs were left. However, isogenic p53^{+/+} control cells gave rise to fewer mitotically non-viable Raju cells, all of which died, probably due to the presence of p53 which is known to kill cells with genomic instability. The basis for the number of Raju cells/NMC is not known at present; this is probably determined by the genetic background of the organism (Sundaram et al. 2004; Rajaraman et al. 2005, 2006).

Neosis in Self-Renewal and Tumor Progression

Raju cells were ~6-10 mm in diameter immediately after birth with a high N/C ratio, displayed characteristic contractile motion or "birth dance" during emergence, detachment and movement away from the NMC (OSM.VC.6). They displayed genomic instability, aneuploidy, and extended, but limited, MLS, probably due to reexpression of telomerase (Kim et al. 1994; Counter et al. 1994, 1998; Meyerson et al. 1998; Gonzalo et al. 2006). The mitotic derivatives of Raju cells had phenotypes and gene expression profiles different from the NMC as indicated by differential display analysis (Sundaram et al. 2004). Raju cells were unique in that they

displayed transient stem cell properties, while their mitotic derivatives had the potential to differentiate albeit in a faulty fashion (See Box 1 for details).

Box 1: Transient stem cell-like properties of Raju cells and the somatic cell properties of the mitotic tumor cell derivatives of Raju cells (Sundaram et al. 2004; Rajaraman et al. 2005, 2006).

Transient stem cell properties of Raju cells:

1. Short cell cycle duration of nascent Raju cells (before they undergo first mitosis) - an indication of lack of G1 phase?
2. Reactivation of telomerase conferring extended mitotic life span.
3. Resistance to genotoxins - Expression of multidrug resistance genes?
4. Are they transiently expressing tissue stem cell specific surface markers? (e.g., CD34⁺ for hematopoietic cells (Reya et al. 2001); CD44⁺, CD33⁻, Lin⁻ for breast cells (Al-Haaj et al., 2003); CD133⁺ for brain cells (Singh et al. 2004), CD20⁺ for skin cells (Fang et al. 2005); CD44⁺, $\alpha 2\beta 1$ hi/CD133⁺ for prostate cancer cells (Collins et al. 2005)).
5. Are they transiently expressing stem cell-specific growth genes? (e.g. Nanog, Oct-4, Wnt, Bmi1 etc.) (Pasque et al. 2003; Chamber and Smith, 2004; Clark et al. 2004)
6. Potential to differentiate, although aberrantly.

Somatic cell properties of mitotic derivatives of Raju cells:

1. Resumption of symmetric mitotic division.
 2. Increase in cell size - Introduction of G1 phase in the cell cycle?
 3. Progressive, but, aberrant differentiation.
 4. Loss of tissue-specific stem cell surface markers due to differentiation during extended mitotic proliferation?
 5. Loss of expression of stem cell-specific self-renewal genes?
 6. Loss of expression of multidrug resistance genes?
 7. They are subject to aging and associated senescence brought about by telomere attrition.
 8. Therefore, they have limited division potential.
 9. Telomere attrition, chromosome breakage-fusion-bridge cycle or genetic stress (e.g., presence of *Ras* oncogene (Takaoka et al. 2004)) will result in senescent phase with MN/PG formation, mitotic crisis, and mitotic catastrophe.
 10. Absence of senescent checkpoints constitutes a built-in mechanism for accumulation of additional mutations via breakage-fusion-bridge cycle, setting in motion the next cycle of S/T-neosis.
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We isolated and cultured individual neotic clones (Raju cells derived from a single NMC). Each neotic clone displayed a characteristic genotype and phenotype, suggesting that neosis gave rise to heterogeneity in the tumor cell population. Only the neotic clones and not the

mitotic clones (non-transformed clones from the same treated plates) (Fig. 2 A-D in Sundaram et al. 2004) grew in soft agar. In addition, the neotic clones that were isolated from cells exposed to 20 μ M etoposide were resistant to 500 μ M etoposide and entered senescent phase and underwent a second round of neosis (S/T-neosis), while the original C3H10T1/2 cells died within a few days after exposure to 500 μ M etoposide. This indicated that neosis is likely the mode of origin of resistant tumor growth after chemotherapy (Sundaram et al. 2004).

We observed primary neosis or P-neosis and secondary/tertiary (S/T-) neosis in several human and rodent cells *in vitro* (Table 1). Human metastatic neuroblastoma derived HTB11 cells underwent three episodes of secondary / tertiary senescence followed by spontaneous S/T-neosis in a progressively non-synchronous fashion, through the course of three years of continuous culture. This indicated that cancer cell are not immortal and they also are subjected to senescence; however, since tumor cells are defective in senescent checkpoint pathway, some of these cells escape cell death via neosis and continued growing as the MLS was extended after interruption by senescent phase (Sundaram et al. 2004).

Thus, new populations of Tumor Rejuvenating Raju cells or TRRCs are repetitively produced in a non-synchronous fashion due to differentiation and ageing of the mitotic derivatives of Tumor Initiating Raju cells or TIRC's via S/T-neosis. At any given time in a tumor tissue, an occasional senescent cell undergoes S/T-neosis in a non-synchronous fashion, producing resistant Raju cells with transient stem cell-like properties and this maintained the continuity of tumor cell lineage. Additionally, since neosis yields viable non-lethal variations of the non-viable polyploidy genome of the NMC, this appears to be the source of tumor cell heterogeneity upon which the process of natural selection may act. This favors the survival of the fittest and leads to the progression of tumors into malignancy.

Neosis and Limits of Extended Mitotic Life Span of Tumor Cells

Neotic clones isolated from C3H10T1/2 cells exposed to etoposide displayed EMLS, probably due to reexpression of telomerase (Sundaram et

al. 2004; Rajaraman et al. 2006; Kim et al. 1994; Counter et al. 1994, 1998; Meyerson et al. 1998). Tumor cells undergo spontaneous senescence *in vivo* and display the senescence marker SA- β -gal sporadically in a non-synchronous fashion (te Poele et al. 2002). Similarly, human adenocarcinoma ACHN cells displayed rare MN/PG cells that underwent S/T-neosis. Human metastatic neuroblastoma HTB11 cells, during continuous culture *in vitro* for three years, underwent three episodes of spontaneous senescence, each followed by S/T-neosis in a progressively non-synchronous fashion, leading to high frequency of cells entering S/T-neosis (Sundaram et al. 2004). As the continuity of cancer cell lineage is facilitated by repetitive cycles of senescence followed by S/T-neosis and EMLS of tumor cells through cancer progression, there is increasing degree of non-synchrony in the onset of senescence. It is likely that additional mutations or epimutations in the cancer cell genome would include the senescence genes and genes that favor neosis. While such alterations in neosis gene(s) may not be favorable for tumor growth, alterations in senescence genes may be conducive for cells to escape senescence. For example, in the case of transitional cell carcinoma (TCC) that represents superficial bladder tumors and invasive bladder cancers, the superficial bladder tumor cells expressed p16INK4A after limited *in vitro* passage and senesced, as did the normal human uroepithelial cells, while all the muscle invasive TCCs contained altered p16INK4A or pRB and bypassed senescence (Yeager et al. 1998). These data suggest that early tumors experience a senescence phase, while malignant tumors might have escaped the senescence barrier probably due to additional mutational or epimutational events in genes that are involved in effecting the senescence program. Most likely, this was facilitated by S/T-neosis. Therefore, one can expect that alterations in the senescence genes, longevity genes and pro-apoptotic genes (Grotewiel et al. 2005; Vijg and Suh, 2005; Morris, 2005; Gami and Wolkow, 2006; Ying et al. 2006) might affect the degree of extension of MLS as the tumor progresses towards malignancy, introducing another level of heterogeneity.

Neosis-like Events in the Literature (Table 1)

A careful literature search revealed that neosis-like events have been reported for more

Table 1: Neosis-like events reported in different cell systems of different species. N* = normal cells, T* = transformed or tumor cells, M* = Mutant cells. (Rajaraman et al. 2006)

Species	Cell type N* or T* Or M*	MN/PGs	P- or S/ T-neosis	Trigger	Consequence	Reference
1. Snail	Primary cells N	Yes	P-neosis	Senescence	Established cell line	Walen, 2004
2. Chicken	Monocytes N	Yes	P-neosis	Senescence	Established cell line	Solari et al. 1965
3. Marsupial	Primary cells N	Yes	P-neosis	Senescence	Established cell line	Walen, 2004
4. Mouse	B16F10 melanoma cells	Yes	S/T-neosis (?)	Methotrexate	Resistant cell growth	Baroja et al. 1998
5. Mouse	Embryonic stem cells M	Yes	P-neosis (?)	Parp-less	Teratocarcinoma	Nozaki et al. 1999
6. Mouse	C3H10T1/2 cells N	Yes	P-neosis	X-ray	Transformed foci	Sundaram et al. 2004
7. Mouse	C3H10T1/2 cells N	Yes	P-neosis	Etoposide	Transformed foci	Sundaram et al. 2004
8. Mouse	1ET1-C3H cells T	Yes	S/T-neosis	Spontaneous	Progression	Sundaram et al. 2004
9. Mouse	1ET1-C3H cells T	Yes	S/T-neosis	X-ray	Resistant cell growth	Sundaram et al. 2004
10. Mouse	1ET1-C3H cells T	Yes	S/T-neosis	Etoposide	Resistant cell growth	Sundaram et al. 2004
11. Mouse	P53-/- MEF/MGB N	Yes	P-neosis	Senescence	Spont. Transformation	Sundaram et al. 2004
12. Mouse	P53+/- MEF/MGB N	Yes	P-neosis	Senescence	Non-viable Raju cells	Sundaram et al. 2004
13. Mouse	P53-/- MEF/129B N	Yes	P-neosis	Senescence	Spont. Transformation	Sundaram et al. 2004
14. Mouse	P53+/- MEF/129B N	Yes	P-neosis	Senescence	Non-viable Raju cells	Sundaram et al. 2004
15. Mouse	L cells T	Yes	S/T-neosis(?)	Arginine	Resistant tumor growth	Wheatley, Persnl communication
16. Armenian hamster	AHL cells N	Yes	P-neosis	X-ray	Transformed foci	Sundaram et al. 2004
17. Rat	REF N	Yes	P-neosis	X-ray	Transformed foci	Sundaram et al. 2004
18. Rat	X-REF23 N	Yes	P-neosis	X-ray	Transformed foci	Sundaram et al. 2004.
19. Rat	Adenocarcinoma cells	Yes	S/T-neosis	Cisplatin	Resistant tumor growth	Martin F, Persnl.communication
20. Mammals	Trophoblasts N	Yes	P-neosis	Senescence (?)	Non-viable Raju-like cells	Zybina et al. 1974, 1979
21. Human	Amniocytes N	Yes	P-neosis	Senescence	Established cell line	Zitcer and Dunnabecke, 1957
22. Human	HT 1080 cells T	Yes	S/T-Neosis	Chemical	Resistant tumor cells	Buikis et al. 1999
23. Human	Breast epithelial cells N	Yes	P-neosis (?)	Senescence	Transformed cell line	Romanov et al. 2001
24. Human	Prostate cancer cell PC3	Yes	S/t-Neosis	Doxotaxol	Resistant cell growth	Marakovskiy et al. 2002
25. Human	Burkitt's lymphoma cells	Yes	S/T-neosis	Radiation	Tumor progression	Erenpreisa et al. 2000
26. Human	Amnion cells N	Yes	P-neosis	senescence	Established cell line	Walen, 2004
27. Human	Amnion cells N	Yes	P-neosis	SV40	Transformed cells	Walen, 2004
28. Human	Adenocarcinoma cells	Yes	S/T-neosis	Spontaneous	Tumor progression	Sundaram et al. 2004
29. Human	FSK cells N	Yes	P-neosis	X-ray	Non-viable Raju cells	Sundaram et al. 2004
30. Human	MRC-5 cells N	Yes	P-neosis	X-ray	Non-viable Raju cells	Sundaram et al. 2004
31. Human	HTB11 cells T	Yes	S/T-neosis	Spontaneous	Tumor progression	Sundaram et al. 2004
32. Human	HTB11 cells T	Yes	S/T-neosis	X-ray	Tumor progression	Sundaram et al. 2004
33. Human	HeLa cells T	Yes	S/T-neosis	X-ray	Tumor progression	Sundaram et al. 2004
34. Human	Colon carcinoma HT116	Yes	S/T-neosis	Doxorubicin	Tumor progression	Sikora E, Personal communication

than a century under different names such as c-mitosis, a-mitosis, or direct mitosis (Boveri 1929; Walen 2002; 2004; 2005), nuclear budding (Zitcer and Dunnabecke 1957; Solari et al. 1998; Walen 2002; Erenpreisa et al. 2000; 2002; Ivanov et al. 2003), sporosis (Buikis et al. 1999), nuclear fragmentation (Zybina et al. 1974; 1979), in a variety of cell systems either spontaneously at senescence or after exposure of tumor cells to genotoxins (Reviewed in Rajaraman et al. 2005, 2006; Table 1). However, this process did not receive the attention it deserves so far. Since the publication of our work on the behavior of neotic clones and its significance in cancer growth (Sundaram et al. 2004), in spite of the initial skepticism due to the absence of other reports (Navalonic et al. 2004), interest in this area has increased considerably as more and more laboratories are beginning to report similar observations (Martin and Solary, 2004; F. Martin, Personal communication, 2004; J. Erenpreisa, Personal communication, 2004; Erenpreisa et al. 2004, 2005a,b; Walen 2004, 2005 and personal communication, and E. Sikora, Personal communication, 2006; Parris 2006; Rajaraman et al. 2005, 2006; L. Reineckie, personal communication). Although this has been reviewed earlier (Rajaraman et al. 2005, 2006), a few points are worth mentioning again with reference to this review: (1) First reference to this process in connection with spontaneous origin of 'immortal' cells from senescent amniocytes was reported by Zitcer and Dunnabecke, in 1957. (2) The reports on the emergence of small mononucleate Raju-like cells from the extraembryonic polyploid

trophoblast cells in the mammalian placenta by Zybina et al. 1974, 1979 are significant from the point of view that this occurs *in vivo* in mammals and implies that this process has been conserved evolutionarily and is ectopically expressed during tumorigenesis (See Table 2). (3) The human colorectal cancer HT116 cells when exposed to doxorubicin displayed the senescent marker SA- β -gal and such cells were capable of undergoing neosis (E. Sikora, 2006, personal communication).

Cell and Molecular Biology of Neosis

Since the significance of neosis has been brought forth only recently (Sundaram et al. 2004; Rajaraman et al. 2005), virtually nothing is known about the molecular events that lead to neosis. However, studies on mitotic catastrophe have been reported for many years. Mitotic catastrophe kills tumor cells, while neosis extends their MLS, and both phenomena are mutually exclusive (Rajaraman et al., 2006; See also Fig. 1). However, they have a common origin in the cellular response to DNA damage, and therefore, the studies on mitotic catastrophe might elucidate some aspects of neosis. With this in mind, we are summarizing the likely molecular events that might lead to successful completion of neosis, with a view to test these assumptions by further experimentation

Both normal and cancer cells can undergo premature senescence, when exposed to genotoxins and when there is inadvertent activation of mitotic signals such as was observed following transfection with the activated Ras

Fig. 1. Fate of cells exposed to genotoxins: Immediate effect of exposure to genotoxins is the arrest of cell cycle progression. Cells with lethal damage will undergo necrotic death immediately or may commit immediate or delayed suicide by programmed cell death or apoptosis.

Adaptation I. Some cells with minimal damage may re-enter cell cycle after some delay and repair of damage, and multiply normally without any immediate phenotypic changes. It is likely that some of these cells may carry epigenetic alterations and undergo neosis after a latent period of accumulation of additional damage to the genome.

Adaptation II. Some cells become tetraploid due to cytokinesis failure. Some of them may commit apoptosis, or undergo mitotic catastrophe due to active mitotic checkpoint; such cells often form micronuclei during death. Some of them may undergo successfully multipolar mitosis, giving rise to aneuploid cells, which may not survive to give rise to clonal population of tumor cells.

Adaptation III. A major fraction of cells enter a premature senescent phase due to genotoxin-induced DNA damage; by about a week or so, they express senescent markers such as SA- β -gal and SAHF in order to suppress tumor growth; they may become polyploid by endomitosis and endoreduplication. Most of them may eventually die.

Adaptation IV. By about second week after exposure to genotoxins, a few of the tetraploid and polyploid cells with genetic or epigenetic alterations in the senescence pathway may undergo neosis to give rise to aneuploid Tumor Initiating Raju Cells (or TIRCs) with transient stemness. These are the precursors of primary tumor growth with extended MLS. They mature into tumor cells. At the end of their limited MLS, they reach senescent phase and undergo S/T-neosis and repeat the cycle of extended MLS, senescence, mitotic crisis and neosis several times, thus rejuvenating the supply of resistant (malignant) Raju cells in a highly non-synchronous fashion. These are termed Tumor Rejuvenating Raju Cells (or TRRCs) (See the text for further details).

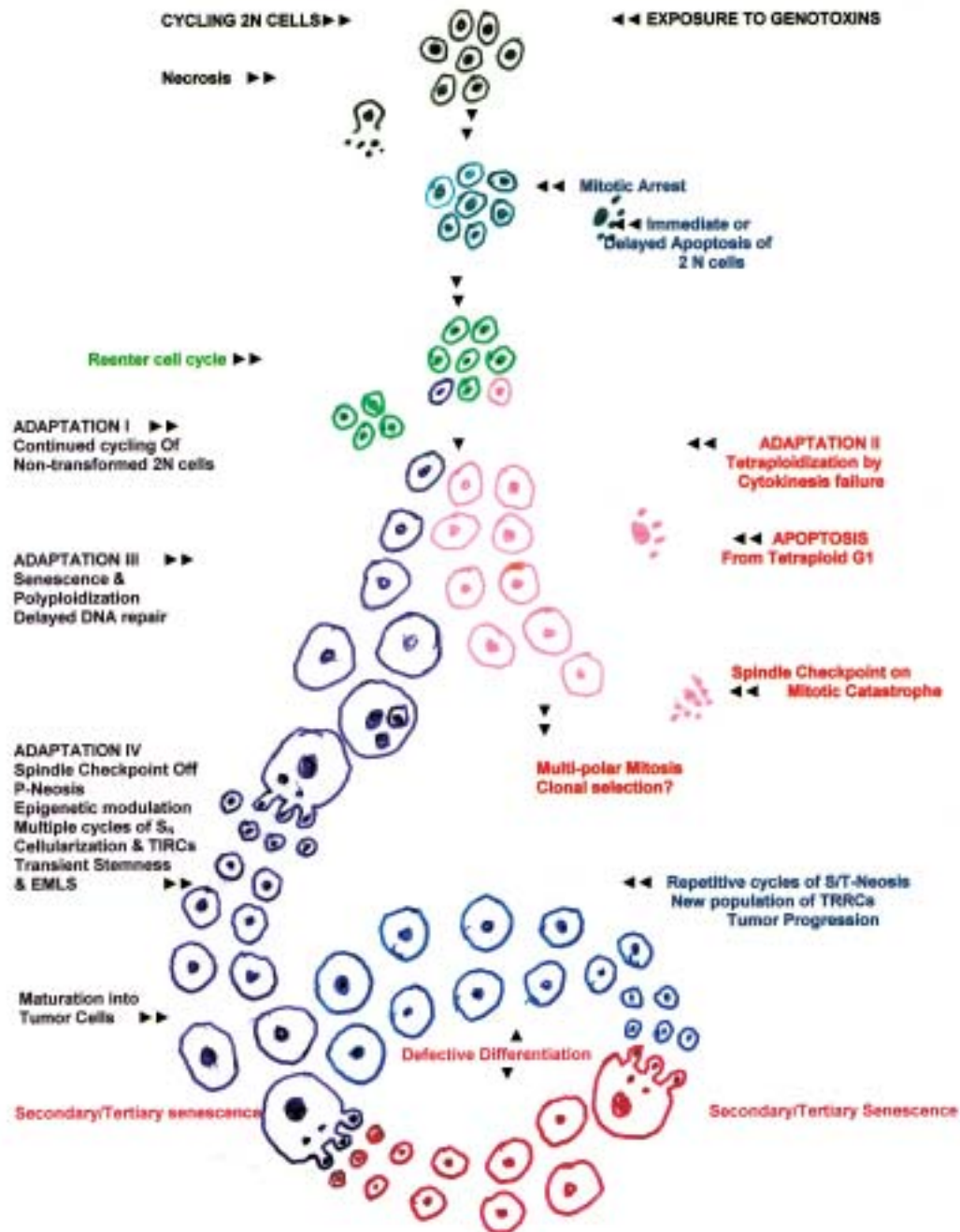


Table 2: Similarities between trophoblast maturation and tumor cell self-renewal (Rajaraman et al., 2006)

<i>Properties</i>	<i>Trophoblast</i>	<i>Tumor cell</i>
1. Subject to ageing and senescence	Yes	Yes
2. Polyploidization by endomitosis and endoreduplication	Yes	Yes
3. Polyploid giant cell undergoes neosis	Yes	Yes
4. Activation of telomerase	Yes	Yes
5. Multiple neotic offspring	Yes	Yes
6. Degradation and migration through extracellular matrix	Yes	Yes
7. Secretion of proteases degrades extracellular matrix	Yes	Yes
8. Invasive properties	Yes	Yes
9. Proteolysis of thrombin receptor	Yes	Yes
10. Stimulation of invasive properties	Yes	Yes
11. Evasion of immune rejection	Yes	Yes
12. Activation of protooncogenes	Yes	Yes
13. Growth control by tumor suppressor genes	Yes. Under normal circumstances	No – Lost during neoplastic transformation
14. MLS of Raju cells or their equivalent	Limited MLS and perish at the end of pregnancy	Limited. Can extend MLS via repetitive S/T-neosis.

gene in vitro (Braig et al. 2005; Braig and Schmitt 2006; Reviewed in Rajaraman et al. 2006). Cells in the absence of p53 or related tumor suppressor function, arrive at G2/M phase and may undergo delayed mitosis, and may die via mitotic catastrophe, often giving rise to small micronuclei, which enclose fragments of chromosomes or genomic DNA, a phenomenon termed mitotic catastrophe. In these instances, spindle checkpoint control is activated during breakdown of the nuclear envelope in preparation for mitosis. At the molecular level, mitotic checkpoint prevents advancement of cells to anaphase by inhibiting anaphase-promoting complex or cyclosome (APC/C). This is accomplished by recruiting checkpoint proteins, including Bub1, BubR1, Bub3, Mad1 and Mad2 to unattached kinetochores, which may inhibit cdc20. This, in turn, will inhibit the recognition of mitotic substrates including securin and cyclin B (Fang 2002; Fang et al. 2006; Sudakin et al. 2001; Tang et al. 2001). During mitotic catastrophe, cells with DNA damage enter mitotic phase by disassembling the nuclear envelope and the spindle checkpoint is activated; however, being unable to successfully complete mitosis, the cells die during the process, often yielding multiple micronuclei that contain fragments of the genomic DNA (Roninson 2001).

However, under normal circumstances if proper alignment of all the kinetochores is achieved, APC/C inhibiting signal is silenced and

APC/C^{CDC20}-mediated ubiquitination of securin leads to activation of its binding to separase. This cleaves the cohesions and allows the separation of sister chromatids and the onset of anaphase. Degradation of cyclin B inactivates Cdk1, which allows the cell to exit mitosis (Reviewed in Wasch and Engelbert 2005).

A subpopulation of cells do not undergo mitotic catastrophe and they remain tetraploid due to the failure of cytokinesis (Andreassen et al. 2003; Borel et al. 2002). It has been generally assumed that rare non-disjunction during bipolar division in diploid cells or multipolar mitosis of tetraploid cells give rise to tumorigenesis, since these will result in unequal distribution of chromosomes to daughter cells resulting in aneuploidy, a phenomenon commonly seen in solid tumors (Hanesmann 1890; Boveri 1929; Nitta et al. 2004; Hardy and Zacharias 2006). Accordingly, a parasexual cycle of polyploidization and segregation of chromosomes has been reported to occur in human aged, tetraploid fibroblasts, which has been assumed to involve multipolar spindle formation and chromosome non-disjunction (Martin and Sprague 1969; Terzi and Hawkins, 1975). However, it should be pointed out that the observations of both Hanesmann (1890) and Boveri (1929) were essentially made in tumor cell populations, which led them to arrive at this conclusion. Since tumor cells have already lost genomic stability, they could tolerate errors in chromosomal distribution

and continue dividing in order to survive (Fisher 1994). Therefore, this does not address the question of origin of aneuploidy. We have recently proposed neosis as the third and most likely mode of arriving at aneuploidy (Rajaraman et al. 2006).

It has been shown recently that structural and numerical chromosome alterations in colon cancer develop through telomere-mediated anaphase bridges and not through mitotic multipolarity. In fact, multipolarity resulted in uneven chromosome distribution to daughter cells that gives rise to gross genomic changes such as nullisomies and non-viable daughter cells, and therefore, rarely contributed to the clonal evolution of tumor cells (Stewenius et al. 2005). All human aneuploid cells (that have chromosome number other than 46) in normal diploid human cell systems that occur during development result in embryonic lethality, except certain combinations of sex chromosomes and hyperdiploid, trisomies 13, 18 and 21, which yield severe birth defects in humans (Hassold et al. 1996; Kops et al. 2005). More importantly, it has been recently shown that chromosome non-disjunction in primary human cells yields tetraploid cells rather than aneuploid cells due to failure of cytokinesis. This demonstrates that tetraploid cells do not directly give rise to aneuploidy and there must be an intermediate step between tetraploid cells and aneuploid progeny of tumor cells (Shi and King 2005). We have demonstrated that such tetraploid cells undergo neosis and give rise to aneuploid Raju cells that matured into transformed cells (Sundaram et al. 2004; OSM.VC.1). Thus, neosis appears to be the intermediate pivotal process between tetraploid cells and the origin of aneuploid tumor cells. It has the potential to give rise to aneuploid or near diploid Raju cells via polyploidization followed by karyokinesis via nuclear budding in the absence of mitotic checkpoint control. Polyploidization is often accompanied by multiple centrosomes, which fuse to form a complex bipolar spindle, thus avoiding multipolarity (Borel et al. 2002; Quintyne et al. 2005). Accordingly, p53^{-/-} MN/PG cells spontaneously gave rise to transformed cells via neosis (Sundaram et al. 2004) and in p53 null mouse cells cytokinesis failure-generated tetraploid cells promote tumorigenesis *in vivo* (Fujiwara et al. 2005). These cells have extended MLS in the absence of senescent checkpoint

controls (eg. P53^{-/-} cells); but they perish in the presence of proper checkpoint controls (eg., p53^{+/+} cells) (Sundaram et al. 2004; Rajaraman et al. 2005, 2006). Thus, p53^{+/+} MN/PG mouse cells did not yield viable TIRCs (Sundaram et al. 2004) and the p53^{+/+} tetraploid cells did not produce tumors *in vivo* (Fujiwara et al. 2005). It is also known that in several human and rodent tumor systems, tetraploidy is the intermediate stage before the genesis of neoplastic growth (Bunz et al. 1998; Andreassen et al. 2001; Borel et al. 2002; Huang et al. 2005; Shackney et al. 1989; Heselmeyer et al. 1998; Ornitz et al. 1987; Cross et al. 1995).

Therefore, we have proposed that senescent cells with tetraploid or higher ploidy genomes have the potential to undergo neosis, creating conditions for an automatic onset of aneuploidy to drive malignancy, if all the conditions for survival of the genome are met with in the resultant Raju cells, with transient stem cell properties and with extended MLS (Rajaraman et al. 2006).

Polyploidy can result either due to endomitosis and endo-reduplication (Zybina et al. 1979) or by cell fusion (Solari et al. 1995). Mad2- or BubR1-depleted cells (the genes involved in spindle checkpoint control) that do not complete cytokinesis remain viable through continued cycles of DNA replication up to at least 32 N (Kops et al. 2004; Michel et al. 2004). Even higher ploidy can be attained in certain cell systems, which can successfully complete neosis (Erenpreisa et al. 2002; Zybina et al. 1979). Polyploidization, in addition to protecting cells from death, confers evolutionary potential by producing cells with non-lethal variations in the genome, upon which the process of natural selection may act (Sundaram et al. 2004; Illedge et al. 2002; Therman et al. 1986; Storchova and Pellman 2004; Rajaraman et al. 2005, 2006). In addition, since spindle checkpoint control is activated after the dissolution of the nuclear envelope (Kops et al. 2004), neosis, by performing karyokinesis via nuclear budding by suppressing the dissolution of the nuclear envelope, appears to be an adaptation by polyploid cells to escape from spindle checkpoint control-induced mitotic catastrophe. It is known that p53 null cells eventually develop polyploidy (Sundaram et al. 2004; Borel et al. 2002) following the formation of multiple centrosomes. It has been reported that the presence of multiple centrosomes can avoid formation of multiple spindle poles by fusion of centrosomes resulting in a complex form of bipolar

spindle (Borel et al. 2002). However, it is not known as to how the process of karyokinesis is carried out in the presence of the nuclear envelope. The question arises that if the complex of multiple centrosomes acts like the spindle pole body of budding yeast by physically associating itself with the nuclear envelope and relocates itself back in the cytoplasm in Raju cells to facilitate symmetric mitosis (Walen 2005). Future studies will have to determine the mechanisms of the ordered movement of newly synthesized chromosomes from the polyploid non-viable genome preferentially to the newly formed nuclear bud. This is a unique property of stem cells that is being displayed by the NMC (Sell 2004; Rajaraman et al. 2005, 2006).

Also, the basis for the number of Raju cells produced by each NMC is not known. For example, in the primary cultures of p53 MEF/MGB cells we observed that the cells underwent spontaneous multinucleation and polyploidization at the end of MLS with multiple giant nuclei present, and often more than one nucleus can produce nuclear buds and yield TIRC. Number of Raju cells / NMC was around 10 to 12 in C3H10T1 $\frac{1}{2}$ cells, ~4 or 5 in HeLa cells, and ~50 or more in p53^{-/-}MGB/MEF cells, but 1 or 2 in p53^{-/-}MEF/129B cells. While it is tempting to postulate that the number of Raju cells / NMC may depend upon the ploidy of the NMC, this remains to be tested. It is very likely in addition to ploidy, the cellular genetic background may be a determining factor in the number of Raju cells/NMC (Sundaram et al. 2004).

Neosis and Epigenetics in Tumor Progression

“The genome functions like a highly sensitive organ of the cell that monitors its own activities and corrects common errors, senses unusual and unexpected events, and responds to them, often by restructuring itself” (McClintock 1986). This statement is likely to reflect the behavior of cancer cells. Recent studies have revealed that genetic mutations alone do not lead to cancer. Evidence has been accumulating on the substantial contribution of pathological epigenetic alterations – non-DNA sequence-based heritable alterations – to the onset of early changes in the process of carcinogenesis, that have been shown to initiate genomic instability, even before gene mutations enter into the process (Egger et al. 2004; Feinberg et al. 2006; Baylin and Ohm 2006). Epigenetic

mutations fall into two main categories: (1) Altered DNA methylation of CpG dinucleotides, both losses or hypomethylation (results in gene activation) and gains or hypermethylation (results in silencing the gene) and (2) altered patterns of histone modifications such as acetylation or methylation, (Jones and Laird 1999; Jones and Baylin 2002; Fraga et al. 2005). Epigenetic Modulation (EM) is thought to be the mechanism by which the genome alters its behavior in response to the environment (Qiu 2006).

EM occurs during all stages of tumor growth from the initiation at the progenitor cells through tumor formation and progression (Fienberg et al. 2006; Baylin and Ohm 2006). During the initiation stages, epigenetic modifications mimic the effect of genetic damage by altering the expression of tumor suppressor genes, thus compromising the tumor suppressor function of the senescence program; for example, silencing of tumor suppressor genes by promoter DNA hyper-methylation and chromatin hypoacetylation, which may affect the expression of diverse genes including p53 (Langley et al. 2002) RB1, p16INK4A, Von Hippel-Landau tumor suppressor (VHL) and MutL protein homologue 1 (MLH1) (Langley et al. 2002; Akai et al. 1991; Jones and Baylin 2002; Herman and Baylin 2003). Global hypomethylation of chromatin leads to chromosomal instability and increased tumor incidence both *in vitro* and *in vivo* (Eden et al. 2003; Holm et al. 2005). EM is responsible for the activation of *Ras* gene in gastric cancer (Nishigaki et al. 2005), and for the activation of cyclinD2 and mpsin in pancreatic cancer (Oshimo et al. 2003; Akiyama et al. 2003).

When such defective cells reach senescent phase and undergo neosis, the NMCs give rise to near-diploid or aneuploid Raju cells; this indicates that neosis must comprise properties of meiosis, mitosis and neosis-specific events. During the extended MLS, Raju cells mature into tumor cells, while also accumulating additional mutational and epigenetic events, which increase the GI load, forcing the cells to initiate the next senescent phase due to mitotic crisis. The onset of senescence is accompanied by several changes in the gene expression profile of the cells: (1) downregulation of mitotic genes; (2) upregulation of anti-mitotic genes (Roninson 2002; Dimri 2005). Re-expression of the ‘immortalizing enzyme’ telomerase is obligatory for the extension of mitotic life span, but not sufficient for neoplastic transformation (Kim

et al. 1994; Counter et al. 1994, 1998; Meyerson et al. 1998; Dong et al. 2005; Gonzalo et al. 2006). Several other meiotic genes collectively called Cancer / Testes (CT) antigens are also expressed in tumor cells and are thought to play a role in transformation (Sagata 1997; Fukasawa et al. 2006), in addition to ectopic expression of stem cell self-renewal genes including microRNAs, notch, oct4, and Bmi1 (Weinberg 1989; Mihich and Hartwell, 1997; Passegue et al. 2003; Chambers and Smith, 2004; Breuer et al. 2004; Clark et al. 2004; Lu et al. 2005) and multi-drug resistance genes (Sarkadi et al. 2004; Dean et al. 2005), among others.

Accordingly, it has been recently demonstrated that exposure of tumor cells to genotoxins results in the translational upregulation of the *cMOS* gene (Kalejs et al. 2006), which is involved in switching cells from mitosis to meiosis II and forcing the cell to undergo reduction division (Tachibana et al. 2000). Constitutively expressed in low levels in untreated tumor cells, expression of meiotic cohesion gene REC8 was also enhanced after irradiation of p53 mutated Namalwa Burkitt's lymphoma cells, along with other meiosis-specific genes DMC1, STAG3, SYCP1 and SYCP3. Expression of these genes reached a peak level during the mitotic arrest phase and was proportional to the endopolyploid cells (Kalejs et al. 2006). Therefore, the most significant event during neosis appears to be the alteration in gene expression profile, which is likely brought about by epigenetic genome/chromatin modulation (EGCM). This will result in the reduction of the GI load, making it possible to produce mitotically competent genomes of Raju cells from the non-viable polyploid genome. Thus, the genes for neosis, which are silent in the genome and which should be active only during the maturation of the extraembryonic trophoblast cells during pregnancy, are ectopically expressed in tumor cells in the absence of p53 or related tumor suppressor genes due to DNA damage. Our data and those of others (Sundaram et al. 2004; Erenpreisa et al. 2002; 2004, 2005; Walen 2002, 2004, 2005) suggest that the genome of such polyploid cells, although not mitotically viable, may undergo EGCM to reduce the degree of GI load to produce mitotically competent TRRCs with EMLS and thus contribute to the continuity of tumor cell lineage in a non-synchronous fashion, while the mitotically non-viable polyploid genome is discarded by the

post-neotic death of NMC. Neosis is repeated several times during the growth of tumors. This implies that global EGCM occurs throughout the life of the tumors, repetitively at least during each neosis, since continuous proliferation will lead to accumulation of gene mutations and epimutations, which may be often detrimental to the cell.

Thus, neosis appears to be the right of passage of a tumor cell. After the P-neosis, a cell cannot revert back into normalcy, except in cases where the alterations in gene expression were brought about by epigenetics alone. Thus, embryonic carcinoma cells derived from teratocarcinomas produce tumors, when injected into appropriate hosts. But, surprisingly, when injected into blastocysts, they can often develop into normal somatic cell lineages (Andrews 2002; Mintz 1978). However, in nuclear transfer studies, the phenomenon of reversibility into normal cells by reprogramming the gene expression profile was not complete when the embryonic carcinoma cell nuclei came from the cell lines with genetic lesions such as over-expression of genes, including Nanog, from the 12p13 region in numerous human carcinoma cells (Belloch et al. 2004; Chambers et al. 2003; Okamoto et al. 1990).

Therefore, we propose that cells with defective senescence checkpoint control(s) tend to undergo polyploidization via endoreplication / multinucleation and after restructuring the genome followed by multiple rounds of neotic S phase (S_N phase), produce daughter Raju cells with EMLS via nuclear budding and asymmetric cytokinesis. This decreases the GI load in the Raju cells, while the non-viable polyploid genome of the NMC is discarded during its post-neotic demise. In the absence of tumor suppressor gene(s), the genome appears to be highly plastic (and tolerant to DNA damage) and responds to the damage and never activates the mitotic checkpoint by keeping the nuclear envelope intact and tides over the crisis by producing several Raju cells with stem cell properties and helps the continuous growth of tumor.

Neosis and the Current Concepts of Cancer

Our observations on the escape of cells from senescence via neosis and the repetition of the cycle of genesis and regeneration of Raju cells interspersed non-synchronously by the senescent phase several times during tumor growth challenge the current concepts of cancer biology.

Table 3: Differences and similarities between Adult Stem Cells and Somatic cells.

<i>Properties</i>	<i>Adult Stem Cells</i>	<i>Somatic cells</i>
1. MLS	Unlimited (?)	Limited
2. Duration of MLS	Longer than organism's life	Longer than organism's life (?)
3. Response to mitotic stimuli	Asymmetric / Symmetric	Symmetric
4. Response to differentiation stimuli	Asymmetric	Symmetric
5. Telomere length	Age-related reduction	Age-related reduction
6. Telomere attrition	Yes	Yes
7. Potential to senesce	Yes	Yes
8. Effect of hTERT transduction	Prolonged doubling potential	Prolonged doubling potential
9. Telomere dysfunction	Mitotic crisis and catastrophe	Mitotic crisis and catastrophe
10. P53-induced DNA repair	Yes	Yes
11. Response to tumor suppressor function	Mitotic crisis and Senescence	Mitotic crisis and Senescence
12. Loss of tumor suppressor function	Escape from senescence	Escape from senescence
13. Mode of escape from senescence	Neosis	Neosis
14. Loss of genomic stability	Initiates neosis	Initiates neosis
15. Epigenetic modulation	Successful completion of neosis	Successful completion of neosis
16. EMLS of P-neosis progeny	Limited	Limited
17. Properties of neotic progeny	Transient stemness, symmetric division, defective differentiation, initiation of tumor, growth	Transient stemness, symmetric division, growth
18. GI load	Increases during EMLS	Increases during GI load
19. Propagation of genetic defects	Via breakage-fusion-bridge cycle	Via breakage-fusion-bridge cycle
20. Decrease in GI load	Achieved via S/T-neosis	Achieved via S/T-neosis
21. Self-renewal in post-neotic cells	Via repetitive S/T-neosis	Via repetitive S/at-neosis
22. Malignancy	Acquired through natural selection	Acquired through natural selection

Firstly, contrary to the current belief that cancer originates via erroneous mitotic division of a cell with genetic damage (Kennedy et al. 1980), neosis appears to be the source of cancer cells (Sundaram et al. 2004). *Secondly*, cancer cells are not immortal; they are still subject to senescence and they appear to escape cell death via senescence due to mutations or epimutations in the tumor suppressor genes that regulate the senescence pathway. *Thirdly*, since non-synchronous rejuvenation of cancer growth by repetitive production of Tumor Rejuvenating Raju Cells (TRRCs) with transient stem cell-like properties appears to be active both in solid tumors and hematological malignancies, this creates the mirage of the presence of constant pool of immortal cancer stem cells with unlimited asymmetric division potential continuously producing tumor cells and also maintaining the cancer stem cell pool. The arguments against the cancer stem cell hypothesis have been described in detail elsewhere (Rajaraman et al. 2005, 2006) and a short summary is given in Table 3.

But for the above differences in the basic tenets of current cancer biology, the neosis paradigm of self renewal is consistent with the various phenomena known to be involved in carcinogenesis including: (1) Epigenetic silencing of tumor suppressor genes; (2) Reexpression of telomerase during neoplastic transformation that confers extended mitotic life span to tumor cells; (3) Reexpression of stem cell gene(s) involved in self-renewal and multidrug resistance genes; (4) Explains the role of senescence-like multinucleated / polyploid giant cells in tumor tissues; (5) Responsible for the production of aneuploid cells and genomic instability; (6) Points to a role for the expression of meiotic genes in tumor cells; and, (7) May be the source of tumor cell heterogeneity, on which the process of natural selection may act. Thus, the primary function of neosis appears to be to fine tune the non-viable, polyploid genome of the senescent NMC with high GI load by global epigenetic genome and chromatin modulation to produce a viable genome with low GI load, and asymmetrically loading the viable genome into the nuclear bud, thus enabling the continuity of tumor cell lineage.

Cancer as a Single Disease of Uncontrolled Growth via Neosis

The classical view is that cancer is a hetero-

geneous group of disorders with unlimited mitotic potential (immortal) and with markedly different biological properties, (Hanahan; Weinberg 2000; Kinzler; Vogelstein 1997; Please also see http://deainfo.nci.nih.gov/Advisory/ncab/sub-bt/NCABReport_Feb05.pdf) which are the result of clonal selection of mutant and/or epimutant tumor suppressor genes and oncogenes (Baylin; Ohm 2006; Friedberg et al. 2006). Thus, almost 200 different types of cancers, as many as the number of different types of cells in the human body, have been recognized, each with its own characteristic developmental signal transduction pathways and with unique mutations in these pathways. Since each type of differentiated cell would reach its maturity via different signaling pathways, each type of cancer might have different molecular abnormalities that will be responsible for the uncontrolled growth of cancer. This will be further complicated by the number of potential proto-oncogenes or tumor suppressor genes in the signal transduction pathways that can undergo genetic or epigenetic alterations thus initiating or promoting cancer growth. Further, the same gene may not carry an identical mutation even within a group of patients with one type of cancer. This makes it very difficult for the modern approach of developing targeted therapy to treat cancers in a highly specific fashion for the individual cancer types and since the anticancer therapy is directed against mitotic tumor cells, in due course tumors become resistant to these drugs (Weisberg et al. 2006; Litzow 2006; Azam and Daley, 2006; Cortes, 2006), probably, due to S/T-neosis.

However, recent data, including ours suggest that tumor cell heterogeneity is due in part to epigenetic variation in the progenitor cells, and the epigenetic plasticity in addition to genetic variation is responsible to drive tumor progression (Feinberg et al. 2006; Baylin; Ohm, 2006). This concept has added significance to ageing since even maternal twins display epigenetic differences as a function of their age and the environment they were brought up (Fraga et al. 2005). This has resulted in great interest in the role of epigenetic mechanisms in the origin and progression of tumors (Feinberg et al. 2006; Baylin; Ohm 2006; Qiu 2006).

Preliminary data indicate that neosis may be the common denominator for both solid tumors and hematological malignancies (Rajaraman et al. 2005; 2006). Up to ~10% of the tumor cells are polyploid giant cells. Since these are the potential

candidates for S/T-neosis to occur, effective elimination of these distinct populations of cells will reduce the chances of further progression of tumors. If one can successfully identify a common molecular step specific for neosis (see below for examples) among different cancer types, one can conceptualize cancer as a single disease caused by genesis and regeneration of Raju cells via neosis from the point of view of therapeutic molecular targeting. The common features of neosis in hematological malignancies, and solid tumors including carcinomas and sarcomas, may include DNA damage response-induced repair or misrepair, DNA polymerase(s) involved in polyploidization, epigenetic genome and chromatin modulation, activation of telomerase, DNA polymerase(s) involved in repetitive neotic DNA synthesis (S_N), karyokinesis via nuclear budding and asymmetric cytokinesis (Sundaram et al. 2004; Rajaraman et al. 2006). This hopefully will result in a reduction in the number of signal transduction pathways that can be altered during carcinogenesis in order to be able to interfere in the process of carcinogenesis. Thus, the design and development of an ideal anti-neotic agent or neociside to block the progression of multiple types of cancers may be simpler than the steps involved in identifying and developing molecular targets dependant on mitotic genes for individual cancer types or individual patients. Additionally, since senescent cells and therefore, neosis may not occur in normal somatic cells active in mitosis, the collateral damage to normal mitotic cells is bound to be highly reduced. Since S/T-neosis occurs frequently in metastatic population of cells, an ideal neocisides may be effective in treating metastatic tumor patients also, an approach that is not currently feasible.

Neosis and Multistep Carcinogenesis

Several theories of carcinogenesis have been proposed so far including: (1) the theory of step-wise accumulation of gain of function mutations in oncogenes and loss of function mutations in tumor suppressor genes (Weinberg, 1989) (2) loss of checkpoint control leading to genomic instability (Hartwell, 1992); (3) aneuploidy-induced genomic instability (Duesberg et al. 2004); (4) senescent checkpoint and telomere attrition (Sharpless; DePinho 2004); (5) over-expression of anti-apoptotic genes (Lyans; Clarke 1997); and (6) a combination of epigenetic and

genetic alterations leading to genomic instability and tumorigenesis (Freidberg et al. 2006; Baylin; Ohm 2006), among others.

Cancer cells seem to exploit the various phenomena listed above at different stages of their evolution into malignancy. Therefore, neosis paradigm of multistep carcinogenesis encompasses all the above phenomena, in which neosis is the source of primary tumor cells, whose growth is characterized by EMLS, accumulation of mutations through the mitotic proliferative phase, on-set of senescence and escape from mitotic catastrophe, only to repeat the cycle of events several times through tumor progression (Sundaram et al. 2004; Rajaraman et al. 2005, 2006). It further assumes that stem cells, embryonic (ESCs), Gonad (GSCs) or tissue-specific adult stem cells (ASCs) or progenitor cells at any stage of differentiation pathway may be subject to tumorigenic mutations and epimutations (Rajaraman et al. 2005, 2006). It is proposed that genetic or epigenetic mutational events should be sufficient to eventually incapacitate mitotic division by increasing the GI load and to initiate the salvage pathway via neosis. In instances where the initial damage is not severe enough to initiate the neotic salvation pathway, the alterations may be fixed during cell proliferation (promotion) and the primary neosis may be delayed until additional epigenetic and/or genetic alterations accumulate and increase the GI load sufficient to impede mitotic division. We consider neosis is the right of passage for the birth of tumor cell, a point of no return to normalcy once the cells have undergone P-neosis. If only epimutations are essentially involved in tumorigenesis, such cells can be occasionally reverted back to normalcy (Sachs, 1986; Lotum and Sachs 2002; Hochedlinger et al. 2004).

The following sequence of events and processes are envisaged to be exploited by cancer cells to survive and multiply in order to avoid cell death and progress toward malignancy (Fig. 1):

- (1) Accumulation of age-dependent epimutations (Fraga et al. 2005; Baylin; Ohm 2006; Freidberg et al. 2006) or endogenous and exogenous DNA damages may result in loss of checkpoint control(s) (Sharpless; DePinho 2004).
- (2) Loss of checkpoint control(s) results in irreversible loss of genomic stability (Hartwell 1992).
- (3) Cell responds to DNA damage and mitotic arrest ensues (Freidberg 2003).

- (4) Some cells may reenter cell cycle after repair or misrepair of DNA damage (Freidberg 2003).
- (5) Telomere attrition may increase the GI load leading to the senescent phase as a tumor suppressive mechanism (Wright; Shay 1992; Hahn 2004; Sharpless; DePinho 2004; Campisi 2005).
- (6) Cells may arrest themselves and become permanently cytostatic and remain dormant for the rest of life after an initial burst of mitosis (Michaloglou et al. 2005).
- (7) May die via mitotic catastrophe (Castedo et al. 2004)
- (8) Or some cells may escape senescence via neosis by becoming tetraploid due to failure of cytokinesis and may proceed to become polyploid via endomitosis and endoreduplication (Sundaram et al. 2004; Andreassen et al. 2006; Rajaraman et al. 2005; 2006;).
- (9) This is attended by repetitive centrosomal division, which may cluster to form complex centrosomes in order to avoid multipolarity (Faivre et al. 2002).
- (9) The chromatin reorganizes itself into a pre-meiotic bouquet configuration facilitating homologous recombination and sister chromatid exchange (Erenpreisa et al. 2002; Chikashigi et al. 2006; Schertho 2001).
- (10) Delayed DNA repair (Erenpreisa et al. 2002; Ivanov et al. 2003) and chromatin modulation (Freidberg et al. 2006; Baylin; Ohm 2006; Rajaraman et al. 2006) reduces the GI load by reexpressing telomerase (Kim et al. 1994; Counter et al. 1994, 1998; Meyerson et al. 1998), CT antigens (Sagata 1997; Tachibana et al. 2000; Old 2001; Simpson et al. 2005; Kalejs et al. 2006), multi-drug resistant genes (Sarkadi et al. 2004) and some stem cell genes (Passegue et al. 2003; Chamber; Smith, 2004; Clark et al. 2004), among others.
- (11) The chromatin returns to interphase configuration (Erenpreisa et al. 2002).
- (12) The resultant daughter genome is copied several times due to ectopic expression of certain meiotic genes during neotic S phase or S_N phase with repair DNA polymerase(s) (Sundaram et al. 2004; Margolis et al. 2005; Rajaraman et al. 2006).
- (13) The nuclear envelope is kept intact in order not to activate mitotic checkpoint and thus avoid cell death via mitotic catastrophe (Kops et al. 2005; Rajaraman et al. 2006).
- (14) Nuclear bud is initiated into which a single copy of the newly synthesized viable genome is asymmetrically loaded in the absence of a metaphase plate formation. This process might occasionally lead to aneuploidy (Sundaram et al. 2004; Rajaraman et al. 2005, 2006).
- (15) Thus, neosis may be the intermediate step between tetraploidy/polyploidy and the origin of aneuploid cells that may be able to survive due to genomic instability-mediated growth advantage.
- (16) The combination of aneuploidy and genomic instability may act as a built in mechanism to introduce chromosome instability by initiating breakage-fusion-bridge cycle (Matzke et al. 2003; Sharpless 2004; DePinho 2004; Freidberg et al. 2006).
- (17) One (or two?) of the multiple centrosomes may be partitioned and packaged during asymmetric cytokinesis and detachment of the daughter Raju cells (Sundaram et al. 2004; Walen 2005; Rajaraman et al. 2006).
- (18) The number of Raju cells / NMC may be determined by the number of centrosomes or ploidy level and the genetic background of the NMC.
- (19) The newly formed cell is detached from the NMC by sequential cytokinesis yielding one cell after another or by delayed cytokinesis giving rise to a synsytium-like post-budding multinucleate giant cell, which will undergo cytokinetic cleavage giving rise to several Raju cells simultaneously (Sundaram et al. 2004; Rajaraman et al. 2005).
- (20) P-neosis may be the point of no return unless the cells carry only epimutations (Hochedlinger et al. 2004). However, they still may face death via neotic catastrophe (Sundaram et al. 2004; Rajaraman et al. 2006).
- (21) The newer population of Raju cells (TRRCs) may display variable resistance to the conditions that induced the neotic event due to chromatin modulation as an adaptive response to the adverse conditions and will be subjected to natural selection (Sundaram et al. 2004; Rajaraman et al. 2006; McClintock 1984).
- (22) The nascent Raju cells may express tissue

- specific stem cell markers, and behave like committed stem cells in that they immediately divide by symmetric mitotic divisions, give rise to mature tumor cells, accompanied by increase in size (Sundaram et al. 2004; Rajaraman et al. 2005, 2006).
- (23) The mitotic derivatives of P-Raju cells form the primary tumor, and lose the stem cell markers during defective differentiation.
 - (24) Tumor cells are subject to natural selection (Nowell 1976).
 - (25) During the EMLS, the tumor cells may encounter additional mutations and epimutations; this will increase the GI load and the cells will enter next cycle of senescence usually in a non-synchronous fashion.
 - (26) Some of the senescent cells will repeat the cycle of polyploidization, and EGCM and since these cells are already defective in senescence checkpoint control(s), some of them will successfully undergo S/T-neosis non-synchronously producing the next generation of TRRCs.
 - (27) This newer lot of TRRCs is also destined to repeat the vicious cycle of extended MLS, accumulation of mutations and epimutations increasing the GI load, leading to senescence and the next cycle of neosis several times during the growth of tumor.
 - (28) Thus, tumor cells in reality are not immortal; they also undergo periodic senescent phase; but they are rejuvenated due to the parasexual somatic reduction division termed neosis.
 - (29) Since the repetitive neosis occurs in a progressively non-synchronous fashion, this creates the mirage of the presence of a constant pool of cancer stem cells with unlimited asymmetric mitotic division potential.

CONCLUSIONS AND FUTURE DIRECTIONS

The current therapeutic protocols target only the mitotic population of tumor cells. This results in undesirable side effects due to the death of normal mitotic cells in the immune system, intestinal epithelial cells, and hair follicles, where adult stem cells are actively involved in homeostasis. Any improvement in the current therapeutic protocols should take into account hitherto unknown differences between normal and tumor

cell division, if one wants to minimize the non-specific killing of normal cells (Pardee 2004). Neosis occurs only in a subpopulation of polyploid senescent cells. We have provided preliminary evidence for a pivotal role of neosis in cancer and enlisted the major differences between neosis, mitosis and meiosis (Rajaraman et al. 2005). Others have confirmed the presence of similar events in the origin of cancer (Zitcer and Dunnabecke, 1957; Buikis et al. 1999; Erinpreisa et al. 2000, 2005; Walen 2002, 2005). Dr. F. Martin's laboratory has confirmed similar events in an *in vivo* rat model system (Personal communication, 2004). Dr. E. Sikora has reported that senescent cells with the senescent marker SA- β -gal can undergo neosis (Personal communication 2006). The fact that polyploid giant cells are ubiquitous in almost all tumor systems suggests a common role for neosis in cancer.

It is possible to identify cancer specific markers for NMCs or Raju cell, against which one can develop anti-neotic agent(s) or neocides, which would be expected to be highly specific for cancer cells and may minimize or even eliminate undesirable side effects on normal cells. A judicious combination of anti-neotic agent and anti-mitotic agent may allow a reduction in the dose requirement of the anti-mitotic agent used, and thus increase the therapeutic ratio. An ideal neocide(s) may be even useful in preventing the on-set of primary tumor growth in high-risk individuals. It is hoped that the above discussion of the significance of neosis in the initiation and progression of cancers will stimulate further studies in this novel and important phenomenon, thus advancing our knowledge towards better understanding of cancer and developing rational treatment modalities.

NOTES

On-line Supplementary material (OSM) can be found at <http://www.medicine.dal.ca/Rajaraman>. All video clips (VCs) are quicktime-Sorensen format with 15 frames/s. In VC 1-5, Images were recorded every 10 min and in VC.6 images were recorded every 5 min. For further details see Sundaram et al. 2004.

VC.1. Primary neosis in a tetraploid MN/PG formed by C3H10T1/2 cells on post-irradiation day 14; 582 KB.

VC.2. Neotic catastrophe in an MN/PG formed due to irradiation of C3H10T1/2 cells; 2,229 KB.

VC.3. Spontaneous S/T-neosis in HTB11 cells. The first half of the clip shows a Raju cell emerging from a cell lying vertical on the centre right of the frame. The second half shows the emergence of a Raju cell from the

cell lying horizontal in the centre of the frame. 1,578 KB.

VC.4. Mitosis of a nascent Raju cell at the lower left of the frame. 1,578 KB.

VC.5. Raju cell maturing into tumor cell with increase in cell mass displays clonogenicity. A group of Raju cells of varying ages are increasing in cell mass and undergoing mitosis giving rise to a colony of the next generation of tumor cells at the right half of the frame. 1,578 KB.

VC.6. Spontaneous neosis in the p53^{-/-}-MEF/mgb cells. 9,873 KB.

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