Horizontal gene transfers and cell fusions in microbiology, immunology and oncology (Review)

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Abstract. Evolving young genomes of archaea, prokaryota and unicellular eukaryota were wide open for the acceptance of alien genomic sequences, which they often preserved and vertically transferred to their descendants throughout three billion years of evolution. Established complex large genomes, although seeded with ancestral retroelements, have come to regulate strictly their integrity. However, intruding retroelements, especially the descendents of Ty3/Gypsy, the chromoviruses, continue to find their ways into even the most established genomes. The simian and hominoid-*Homo* genomes preserved and accommodated a large number of endogenous retroviral genomic segments. These retroelements may mature into exogenous retroviruses, or into functional new genes. Phages and viruses have been instrumental in incorporating and transferring host cell genes. These events profoundly influenced and altered the course of evolution. Horizontal (lateral) gene transfers (HGT) overwhelmed the genomes of the ancient protocells and the evolving unicellular microorganisms, actually leading to their Cambrian explosion. While the rigidly organized genomes of multicellular organisms increasingly resist H/LGT, de-differentiated cells assuming the metabolism of their onto- or phylogenetic ancestors, open up widely to the practice of H/LGT by direct transfer, or to transfers mediated by viruses, or by cell fusions. This activity is intensified in malignantly transformed cells, thus rendering these subjects receptive to therapy with oncolytic viruses and with viral vectors of tumor-suppressive or immunogenic genetic materials. Naturally formed hybrids of dendritic and tumor cells are often tolerogenic, whereas laboratory products of these unisons may be immunogenic in the hosts of origin. As human breast cancer stem cells are induced by a treacherous class of $CD8^+$ T cells to undergo epithelial to mesenchymal (ETM) transition and to yield to malignant transformation by the omnipresent proto-ocogenes (for example, the *ras* oncogenes), they become defenseless toward oncolytic viruses. Cell fusions and horizontal exchanges of genes are fundamental attributes and inherent characteristics of the living matter.

Contents

1. Introduction: the plasticity of ancient genomes

Proto-spheroplasts of archaea and prokaryota. The primordial cells presumably lived as cell wall-free enveloped proto-spheroplasts (1-3). The cell envelopes consisted of one or two layers of selectively permeable phospholipid membranes. The prokaryotic and archaeabacterial genomes existed as chromosomes not packed into nuclei. The primordial prokaryotic and archaeabacterial proto-spheroplasts vertically transmitted their genomes to their progeny. These units also released endogenous genomic fragments, and were infected, or transfected, by exogenous genomic segments entering them horizontally (laterally) (HGT) (4-6).

Cell membrane phospholipids distinguished archaea from prokaryotes. It is firmly believed that well over three billion years ago archaea and prokaryotes (eubacteria) co-existed. These entities presumably originated from a common ancestor diverging and descending into these two cell lines. The development of cell membrane phospholipids (7) with different glycerol phosphate backbones distinguished the first archaeons and protobacteria. The archaea-specific glycerol-1-phosphate (G-1-P) dehydrogenase and its gene *egs*A

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(enantiomeric glycerophosphate synthase, first identified in *Methanobacterium thermoautotrophicum*) are structurally different from the prokaryotic G-3-P dehydrogenase. The only overlapping feature of the two enzymes is their nicotinamide adenine dinucleotide phosphate-(NAD)-binding regions (8).

The ancient primordial cells from their inception on were programmed for symbiosis and fusion, or for hostile take-over by phagocytosis. The vertically transmitted genomes defined the species. Inheritance by vertical gene transfer is a most highly regulated process. However, intra- and extragenomic retroelements especially the retrotransposons are able to readily intervene. Next to the genome, epigenetic elements efficiently, but reversibly, influence gene expressions. DNA methylations by DNA methyltransferases occur within the cytosine preceding guanine (CpG) oligodinucleotides. In the neucleosomes, the terminal ends of histone octamers are subjected to acetylation or methylation, events that either activate or suppress gene transcription. Obliquely from the side horizontally (laterally) presented genes intrude. In archaea and prokaryotes, the naked DNA transgresses the cell membrane of the proto-spheroplasts, encodes its own enzymes, penetrates the chromosomes not protected by a nuclear membrane, and through its own 'cut & paste' enzymic activity inserts itself into the genome. The young evolving genomes were of high plasticity and remained disposed for the release of full genes, and apt for the voracious acceptance of the arrival of intra- and interspecies genomic sequences ready to be promptly inserted and incorporated. Thus, the ancient genomes might have been quite promiscuous. This may be referred to as the plasticity of the genomes. Extremely active gene-sharing network ruled the evolution of early archaea and prokaryotes. The German 'Eigenspektrum Analysis' revealed that approximately 81% of the genes in each genome participated in mutual lateral gene transfers at various points in the time scale of the long evolutionary history of these microorganisms. The incorporated genes were expropriated for vertical inheritance (6,9).

Eukaryotes emerge. The origin of the first eukaryotic cells is unknown. Horizontal (lateral) transfers and exchanges of genetic material (HGT) occurred between the co-existing cell lines. Most of the exchanged genes remained conserved throughout evolution and persist up to the present. It has been considered that in addition to the common ancestor of archaea and prokaryota, a second separate common ancestor existed for the eukaryotic cells; thus three primitive cell lines (archaea, prokaryota and eukaryota) might have co-existed (2,3). The ancestor of the eukaryotic cell line was the chronocyte (10), neither an archaeon, nor a prokaryote. The chronocyte acquired archaeal and prokaryotic genomic sequences either by HGT, or by phagocytosing archaeal and prokaryotic protocells (10). Thus the eukaryotic genome may be viewed as a mosaic of the original eukaryotic genes and acquired archaeal and eubacterial (prokaryotic) genes (11,12). The early eukaryotic nucleus displayed strong archaeabacterial features, whereas the mitochondria of the eukaryotic cytoplasm are that of alpha-proteobacterial origin (13). The first eukaryotes existing 1.5 billion years ago must have been anaerobic and fungi-like (14). The ancestral photosynthetic eukaryotes acquired plastid organelles from engulfed cyanobacteria; algae and green plants descended from this ancestry (15-18). After the disappearance of mitochondria or plastids, mitochondrial and plastid genes persist in the nucleus of their hosts (18). A chimeric gene collection characterizes the genome of the photosynthetic protist *Euglena gracilis* deriving from photo-autotrophic and anaerobic flagellar kinetoplastid ancestors and endosymbionts (18-20). The Calvin-cycle glyceraldehydes-3-phosphate dehydrogenase gene of *E. gracilis* derives via organelle-to-nucleus transfer from eubacterial endosymbionts, after undergoing duplications (21).

As to the origin of the first eukaryotic cells, it was proposed that one ancestral line was photosynthetic due to the possession of endosymbiotic plastids deriving from cyanobacterium-like prokaryotes (see above). The anti-phage proteins of *E. coli* groEL and groES (large and small), that inhibit phage morphogenesis, showed up in the thermophilic cyanobacterium *Thermosynecoccus* (22-24). Endosymbiotic cyanobacteria transferred the ancestral groE genes into phototrophic eukaryotes. Another ancestral line, the zooflagellate eukaryotes, possessed neither plastids, nor mitochondria; they were anaerobic and not photosynthetic (Archaezoa; Archamoeba; Metamonada) (25,26). Archaezoa were deprived of peroxisomes and Golgi dictyosomes (stacked branching cisterns) and possessed only one centriole (unikont), whereas a tetrakont operates hydrogenosomes, Golgi dictyosomes and three anterior and one posterior, or two divergent pairs, of centrioles (Metamonada). Further complicated subdivisions of primitive eukaryota are directed by the possession of Golgi dictyosomes with or without striated roots, and among other features, if their mitochondria displayed discoid or tubular cristae. HGTs fundamentally altered the structure of the Darwinian Tree of Life (TOL) (15,27,28).

In contrast to the theory that archaeabacteria and eukaryota are sisters diverging from a common ancestor (29), it has been repeatedly proposed that the ancestral eukaryotic cells were formed by the fusion of a primordial prokaryotic (eubacterial, mitochondrium-donor; cyanobacterial, chloroplast plastid-donor) and an archaeal proto-spheroplast (30-33). The surplus of chromosomes carrying genes and operons required intracellular compartmentalization for the storage and co-operative functioning of the genomic overload (34). Thus nuclei within nuclear membranes and cytoplasmic organelles of eukaryotes were formed or acquired. Examples of acquired cytoplasmic organelles of eukaryotes are the mitochondria (deriving from engulfed alpha-proteobacteria), and chloroplasts (deriving from engulfed cyanobacteria) (30). Secondary and tertiary endosymbioses followed (15). Large numbers of mitochondrial and chloroplastic genes were transferred into the nuclei of the complex eukaryotic host cells (13,16). For example, apicomplexan parasites (eimeria, plasmodia, toxoplasma) possess green alga-derived plastid organelles (35-37). Many of these host cell genes at the evoltionary level of algae are inserted into their viruses and remain functional in their new hosts, the viruses, as well. For example, the eukaryotic phytoplankton microalga *Emiliana huxley*i transferred seven host cell genes (voluntarily, or was forced to do so) into its large DNA virus EhV. The authors state: 'ancient viruses controlled the complex metabolic pathways in primitive eukaryotic cells' and the acquisition of host cell genes by the virus helped the virus 'to stay ahead of their

closest relatives' (and competitors) 'in the great evolutionary race for survival' (38).

Viral mediators of gene exchanges and cell fusions. The existence of a pre-cellular autocatalytic nucleic acid network co-existing with amino acids has been envisioned: those were the 'Virus World' with viral hallmark genes circulating among viruses, but not part of cellular genomes, which appeared much later (39); and the 'RNA World' in which ribozymes and ribonucleoprotein enzymes and peptides/proteins co-existed (40). Some of the genomic segments of prokaryotes and archaea are recognized now as bacteriophages, latent but lysogenic, lytic, temperate and fusogenic. In the eukaryotes, these laterally transferred genomic sequences have become their endogenous and exogenous viruses, including those of the ancestral unicellular eukaryotes (algae, fungi and protists), and those of the multicellular plants and animals (41). Because the frequent horizontal (lateral) exchanges of genetic material (HGT) among these three ancestral cell lines was superimposed on the vertical descent of inherited genomes, the construction of a Darwinian TOL based on the assortment of exclusively vertically transferred genes only, remains unresolved and riddled with unexplained puzzles and errors (27). Even the small subunit tRNA-based TOL may fail to depict the interrelationship of diverging lineages of the early hyperthermophilic archaea (42). If a pre-cellular Virus World existed (39), 'selfish viral genomes' might have contributed to the generation of cells in order to replicate and preserve their genomes (43). Once protocells have come to existence, the cell membranes and cell walls became the battle grounds between the virus and the cell (43).

Acantamoebae harboring the mimiviruses (mimicking microbes), and the mimiviruses harboring their virophage, the circular dsDNA 'sputnik,' defy classification in any TOL (44-46). Are they intracellular bacteria confined to obligate cell parasitism due to gene losses, or are they large viruses on their way of gaining independence due to HGT from their cellular hosts? In the same biological perspective: what are chlamydia (*Chlamydia trachomatis*; Virus der vierten Geschlechtskrankheit, lymphogranuloma inguinale venereum; Trachomeinschluss; Psittakosisvirus)? These questions posed decades ago (47,48) remain unanswered (49).

Phages are recognized either as infectious viruses lysing bacterial host cells, or vectors of bacterial genes practicing transfections among bacterial cells. This author emphasizes the co-existence of fusogenic phages with primitive proto-spheroplastic entities of bacteria, namely the *Mycoplasmataceae* (50,51). Proto-spheroplasts of an aged *E. coli* culture passed filters of >350-600 μm pore sizes. Cytoplasmic units in the filtrates fused and regenerated into vegetative bacterial cells (47,52-54). The cytoplasmic units resembling proto-spheroplasts of the aged *E. coli* culture contained dark round granules originally referred to as 'nuclear material' (Kernsubstanz) (47,52). However, these dark cytoplasmic granules did not resemble bacterial chromosomal fragments. The dark granules, shown in references (33,48) resembled the fusogenic phage of the mycoplasma *Acheloplasma laidlawii*. This author proposed that the first eukaryotic cells were formed by the fusion of prokaryotic and archaeabacterial proto-spheroplasts mediated by a fusogenic phage, the ancestor of the *Acheloplasma laidlawii* L3, or a similar and related, fusogenic phage (33,48,49).

Cell divisions. Of the two major archaebacterial phyla, *Euryarchaeota* and *Crenarchaeota*, the crenarchaeota lineage possesses proteins, which are the products of the cell division genes cdvA, cdvB and cdvC, in the form of a three-gene cdvABC operon (54). These gene product proteins are homologous to the protein sorting genes of eukaryotes, the ESCRT-III and CHMP1B gene product proteins (endosomal sorting complex required for transport; charged multivesicular body protein) (54,55). Sharing these conserved proteins indicates that crenarchaeota and eukaryota derive from a common ancestor diverging into these two cell lines; or that horizontal (lateral) genomic transfers and insertions (HGT) occurred between the ancient crenarchaeal and eukaryotic cell lines; or that the first eukaryotic cells emerged from the fusion of prokaryotic and creanarchaeal proto-spheroplasts. This latter event is what this author favors. Of the three classes of crenarchaeal ESCRT-III-like subunits, one subunit interacts with the Vps4 protein (vacuolar protein sorting) in a manner closely related to the interaction between the human ESCR-III and Vps4/Vps20 proteins (54). Of note, prokaryotes initiate their cell divisions with the FtsZ protein filaments (filamentous tubulins with cytokinetic Z rings forming sheets and cyto-skeletal septosomes with affinity to guanine triphosphatase and exhibiting nucleotide binding sites), but do not possess the cdv operons (56-59). Thus, the resemblance of the eukaryotic ESCRT/CHMP gene products to cdv proteins should have derived from a source other than prokaryotic.

The concept of HGF in itself is based on assumptions on inferred archaic events, which are not documented (as yet) experimentally (60). It may be an exaggeration to refer to the HGT concept as a computer-generated 'fallacy'. Therefore, this author proposes experimental approaches to the problem. Events of fusion between crenarchaea and eubacteria/prokaryota could possibly be reproduced in the laboratory by attempting the fusion of extant prokaryotic and crenarchaeal proto-spheroplasts mediated by a fusogenic phage, like the phage L3 of *Mycoplamataceae* (33,49).

Gemmata obscuriglobus. Members of the phylum *Planctomycetes* compartmentalized their chromosomes into nucleoids surrounded by a double-membrane envelope. The key bacterial cell division protein FtsZ (see above) is not operational in planctomycetes. The cell division process of planctomycetes consists of budding and transfer of chromosomal DNA in a naked nucleoid into the bud. In the bud nucleoid membranes are formed only after the transfer of the mother cell's naked nucleoid into the daughter cell (61-63). Thus the cell division process of *G. obscuriglobus* differs from that of archaeons, prokaryotes, and eukaryotes. However, the obscuriglobus genome is that of a prokaryotic one with single eukaryotic genes (integrin-α, α-trypsin inhibitory protein) and archaea-bacterial genes (the tetrahydromethanopterin-dependent enzymes) inserted through ancient HGT (64,65), but not through the fusion of entire prokaryotic and archaeabacterial or eukaryotic genomes. The planctomycete genome is unique without unusally large number of archaeabacterial or eukaryote genes. Further compartmentalization of the planctomycete

cytoplasm includes RNA and ribosome-like particles bounded by a single membrane (63). Strains of *G. obscuriglobus* withstand large doses of ultraviolet and ionizing radiations (66) matching the radioresistance of *Deinococcus radiodurans* (48). Newer Siberian isolates of planctomycetes (67) reveal further bio-morphological features of these distinct bacteria evolving monophyletically towards a nucleated microorganism.

The subject matter of this report. Virally mediated fusion of ancient archaeabacterial and prokaryotic proto-spheroplasts is offered as a theory for the origin of the first eukaryotes. The possible reproduction of these ancient events is testable in the laboratory. This report will review genomic features and nuclear organization of the chromosomes of the 'multinucleated bacterium', the planctomycete, *Gemmata obscuriglobus*; some ostentatious examples of ancient HGT among distant entities of species and genera, which were conserved throughout evolution, with special attention to genes of bacterial (*Tenacibaculum*) origin in the metazoan *Nematostella*; the insertion of the entire *Wolbachia* genome into the genome of insects; and the acquisition of those genes via retroelements/chromoviruses by the first cartilaginous fishes (gnathostomata sharks) that encoded the entire adaptive immune system. As in sarcomagenesis of the entire animal world, in the fused oncogenes/oncoproteins of the human entities of these tumors as yet undiscovered causative retroviruses may be hidden. Further subject matters for review are naturally occurring cell fusions and genomic fusions of malignant cells (melanoma cells) with healthy host cells (macrophages) in the service and for the advantage of the malignant cell, and fusions of healthy cells with healthy cells (myelomonocytes, fibroblasts, myocytes, trophoblats) in the physiology of the host. Several paragraphs are devoted to the story of natural hybridoma formation in murine and human lymphomas The principle will show through that de-differentiated eukaryotic cells, especially malignantly transformed cells, regain the plasticity of the anient genomes, become receptive to genomic insertions, lose and replace genes, and re-establish the ancient cells' disposition for forming unisons by fusions of cell membranes, cytoplasms, nuclei, and genomic segments (genes). The fusion oncogenes-oncoproteins of human sarcoma cells will be used as an example.

Discussion will include a brief review of dendritic cell-tumor cell fusion products for vaccination against human malignancies. When in the laboratory mature dendritic cells (DCs) and inactivated tumor cells are fused, the DCs express tumor antigens in an immunogenic manner. The vaccinated host responds with the generation of major histocompatibility-(MHC)-restricted immune T cells, even in the cases of established metastatic tumors. Complete remission (CR) inductions are rare, but long stabilizations of disease (progression-free extended survivals) are frequently achieved. In contradiction, when the tumor-bearing host's DCs engulf apoptotic or necrotic tumor cells *in vivo*, these unisons express the tumor antigens in a tolerogenic manner. Regulatory T cells and myeloid-derived suppressor cells (MDSC) arise for the recognition of the tumors as 'self' and in the defense of the tumor from rejection. In most tumor-host relationships the tumor overcomes the host, unless external interventions convert tolerance to rejection. Stem cells of the human breast parenchyma undergoing epithelial to mesenchymal (ETM) transition under the effect of a unique class of overstimulated $CD8^+$ T cells, yield to the omnipresent proto-oncogenes (like the *ras* proto-oncogenes) and transform into a malignant clone. The initiator T cells of the process committed a high treason against their host and therefore they should be referred to as traitor/transforming T (T/T T) cells.

2. Horizontally (laterally) transferred interspecies genes

Retroelement insertions. The long terminal repeat (LTR) retrotransposon of the fission yeast *Schizosaccharomyces pombe* encodes an integrase (INT) and targets the *pol* genes for its integration (68). The LTRs and coding sequences of the Tf1 (transposon fission) retrotransposon also encode a reverse transcriptase and a protease. The C terminal chromodomain of the integrase of this Tf1 LTR retrotransposon is the one that binds to the N-terminal tail of chromosomal histone H3 (68).

The ancient retroelements, Ty3/Gypsy are the ancestors of chromoviruses. The 3rd ORF (open reading frame) of the drosophila gypsy encodes the Env protein converting the vertically transmitted retrotransposon to a horizontally spreading retrovirus (68a). One of the chromoviral ancestors, the one in plants, descends to green and red algae (69,70). Plant chromoviruses undergo strict vertical transmissions (69). However, in metazoans, acquisition of *env* genes and encoded Env proteins allow horizontal spread of chromovirales (*Metaviridae*) (71,72). Metaviridae form the chromodomain-containing genus of chromoviruses (72). The protozoan phylum *Cercozoa* (zooflagellates, see above) possesses ancestral LTR-retroelements. The Ty3/Gypsy branch emerged prior to the divergence of protostomes (the invertebrate bilaterians with ventral nerve cords: mollusks, annelids, arthropod insects) and deuterostomes (the enterocoelomate hemichordata, cephalochordata, amphioxus, protochordata tunicates, the ascidian *Botryllus*, and vertebrates) (73-75). Of note, the CUX/CASP (cut-like homeobox-1; conserved alternative-splicing product) genes directed early vertebrate evolution. However, in the lower chordate amphioxus (*Branchiostoma floridae*) the CUX/CASP genes are of protostome derivation (76). The majority of the gypsy LTR-retrotransposons is related to those of fungi, implying ancient events of HGT between ancestral phyla. On the other hand, from many taxa gypsy LTR-retrotransposon lineages, that were existent in their ancestors, were lost. The chromoviruses were 'inexorably driven to extinction' in echinoderms and urochordates, after these genes were horizontally transferred to fungi, plants and vertebrates (77).

The targeted integration of mobile retroelements leads to their enrichment. The chromodomains of chromoviruses are within the C termini of their integrases. The chromoviral chromodomains recognize the epigenetic mark, histone methylation. The fungal retrotransposon MAGGY (of the rice blast fungus *Magnaportha grisea*) interacts with histone H3 dimethylated and trimethylated sites K9. It perpetuates the heterochromatic mark and its epigenetic modification, for example triggering iRNA pathways for post-transcriptional gene silencing (78). The Ty5 retrotransposon of *S. cerevisiae*

integrates preferentially in the heterochromatin of the telomerase loci and to proteins encoded by the *sir* genes (silent information regulators) (79). Transposons DRE1 and TD3 (directly repeated elements; transposon 3 *D. discoideum*) of *Dictyostelium discoideum* integrate next to tRNA genes (79). Of the drosophila retrotransposons, *gypsy* does, and *copia* does not encode an envelope protein. However, the gypsy retrovirus may incorporate the *copia* genome (79). The Ty3/Gypsy retrotransposons contain a conserved GPY/F domain within their integrases, which was acquired by several descendant vertebrate retroviruses (77,80) (G, glycine; P, proline; Y, tyrosine, F, phenylalanine). The three proteins, the Gag-Pol polyprotein, the protease and the GPY/F module characterize most of the classes I, II, III *Retroviridae*. According to this classification, the origin of the class I retroviruses predates the split of fungi, plants and animals. Three polyphyletic ancestral Ty3/Gypsy lineages gave rise to the three classes of both endogenous (vertically transmitted DNA proviruses) and exogenous (horizontally transmitted enveloped viruses) *Retroviridae* (77). The split between the three Ty3/Gypsy lineages occurred before the protostome-deuterostome divergence (73,81) (see above). It is to be seen if the notorious retroviral diversity of the seven genera Alpha, Beta, Gamma (the ancient class I), Delta, Epsilon (sarcomagenic fish retroviruses), Spuma- and Lentiviridae (77), will follow the lineages of Ty3/Gypsy derivation from such triplicate rootings.

Retrotransposons are able to insert themselves into the genome of large DNA viruses. The Marek herpesvirus harbors a genomic segment of a reticuloendothelial retrovirus. The insect baculovirus expresses the copia and the ted (gypsy family) retrotransposons. Ted *gag*, *pol* and *env* genes assemble to encode retrovirus particles with reverse transcriptase in baculovirus-infected hosts (79).

The human genome project reveals the presence of ancient genes of bacterial and archaeal origin in the human genome. Of 113 such incidents, 28 were submitted to phylogenetic analysis by polymerase chain reaction. These genes are proved to have descended through common ancestry, as they were inserted into the genomes of ancestral species early in evolution before the emergence of vertebrates, and have become vertically transferred genes (82).

However, the simian and hominoid genomes remained open for the entry of newly acquired retrotransposons and retroviruses. Almost half of the mammalian genomes derived from ancient transposons (DNA retroelements) (83). Somewhat more than 8% of the human genome was formed by retroviral DNA-proviral insertions (84,85). Due to mutations and deletions most of the inserted retroelements are non-functional. The first human endogenous retrovirus' (HERV-K) proviral integrations occurred in catarrhines (Old World monkeys, ancestors of apes and hominoids-humans) 45 million years ago; the last insertion occurred about 400,000-200,00 years ago (83). These are the testicular germ cell tumor-associated HERV-K(HML) variants, of which the TRIM5 and APOBEC G3-resistant, but APOBEC F3-susceptible HML-2 provirus was mapped to 22q11.21, from where it encodes the Rev protein. (Abbreviations: hemolectin of drosophila, related to human von Willebrand factor; tripartite motif; apolipoprotein B mRNA-editing enzyme catalytic; regulatory RNA-binding protein/factor viral determinant for TRIM5 recognition, related to Rev regulatory virion protein of HIV-1 human immunodeficiency virus-1). The cited reports (83,85) provide an extensive list of historical references. HERV-K insertions occur in gene-rich transcription units, or outside transcription units. Proviruses disrupting gene function can be eliminated by 'purifying selection' (85). Activated endogenous retroviruses are not inert. Activation of an HERV-K in melanoma cells increases malignancy and helps the cells escape from immune surveillance (86).

There is a 'criminal collusion' between retroviruses and other unrelated viruses in the same host (49,87). Herpesviruses, especially EBV and HHV-8/KSHV (Epstein-Barr virus; human herpesvirus 8/Kaposi sarcoma herpesvirus) coexisted with retroviruses in Africa for millennia, but without interference. HHV-8 activates endogenous type C retroviruses in primary effusion lymphoma cells (88) and in Kaposi sarcoma cells (49). The monograph (49) shows the co-existence of herpes- and retroviral particles in KS cells (see below). Even lymphocytic choriomeningitis virus could recombine with the type A endogenous intracisternal retrovirus and have its genome fused with the retroviral provirus integrated into host cell genomes (89).

The origin of genes, which encoded the entire adaptive immune system. In cartilaginous gnathostomata fish MHC molecules and T/B cell receptors capable to undergo somatic hypermutations replaced the variable lymphocyte receptors (VLR) composed of leucine-rich repeats (LRR), that remained still functional in the extant *Agnatha/Cyclostomata* (lamprey and hagfish) (49,90-92). The LRRs are considered to be members of the Toll-like receptor (TLR14ab) family (92). The VLRs could generate diversity of their antigen-binding sites: the leucin-rich repeats so generated recognize and respond to antigens (93,94). The variable (hypervariable) concave surface of the horseshoe-shaped LRRs bound antigens (91). This was an adaptive immune response, however different from immunoglobulins (95). Even though the diversities of both immunoglobulins and VLRs are generated by gene rearrangements, the VLRs are not immunoglobulins (91,95). Thus two separate systems were generated in the Cambrian sea 600-500 million years ago with some overlap, but with very distinct end products (LRR and immunoglobulins).

Farther down on the scale, the amphioxus/lancelet operates Toll-like receptors (TLR), NOD-receptors (nucleotide-binding oligomerization domain), complement-like molecules, opsins and tumor necrosis factor (TNF) (96,97). The amphioxus possesses fifteen homeobox (Hox) genes. These are the ancient genes generated in anemones, amphioxus, hydra and jellyfish (98), which re-appear in vertebrates in multiple duplicated copies, as 3 or 4 paralogs of the original amphioxus genes (96,97). The genome of the cnidarian anemones encodes B factor, C3, and mannan-binding protein-associated serine proteases of the complement system (99). To the large sets of TLRs, NODs, lectins and complement pathways, the echinoderm sea urchins added genes and gene product proteins with recognizable homology to vertebrate recombination activating genes (RAG1 and 2) (100). These gene product proteins (enzymes) certainly did not act in the sea urchin on a V(D)J (variable, diversity, joining) system, inasmuch that system was non-existent (not found as yet) in that host.

The adaptive immune system evolved in the ancient gnathostomata sharks (the now extinct *Placoderms*; the extant *Carcharhine* sharks) from ancient genes pre-existing in a dispersed distribution in sponges (*Demospongiae*), mollusks, proto-cephalo-chordates (amphioxus, *Branchiostoma*), anemones (*Nematostella*), and echinoderms (sea urchins, *Strongylocentrotus*) (49,101-105). The shark recombination activating gene RAG 1 domain 4 protein shows homology with bacterial and bacteriophage (phage λ) integrase, and RAG 2 resembles gram-negative bacterial integration host factor (IHF) proteins (106-108). These ancient genes traveled through sea urchins, which were not vertical ancestors of the sharks, before they reached the gnathostomata sharks. As to the T cell receptor (TCR), the three-dimensional structures of its α/ß chain immunoglobulin domains are related to shark immunoglobulin variable light and heavy chain V(H) domains (109). In the post-Cambrian sea, the ancestral MHC (major histocompatibility complex), V(D)J, RAG and RSS (recombination signal sequences) genes re-assembled, aligned and have begun working for the first time in unison (49). The gene product proteins are derivatives of the ancient retroviral enzymes that cut dsDNA and insert proviral DNA segments in a 'cut & paste' fashion (110,111). The AID-APOBEC enzymes (activation-induced deaminase; apolipoprotein B-editing enzymes catalytic) are active in the lymphocytes of the lamprey (112) and remain active anti-retroviral agents in the mammals, including simians and *Homo* (49). The arrival of these genes into the primitive cartilaginous (chondrichties; *Chondrichthyes*) fish was presumably through nutritional routes (jaws crushing sea urchins; lymphoid tissues surrounding the alimentary canal accepting the insertion of new genes); or by direct retro-element (retrotransposon, retrovirus) genomic insertions (overcoming suppression by APOBECs). Villarreal presents strong argument that it was chromovirally mediated retro-transposon insertions that endowed the ancient sharks with the full genetic machinery of the entire adaptive immune system (specific antibody synthesis in B lineage cells; configurational changes of the TCR enabling it to recognize MHC-restricted and presented non-self antigens) (113).

Such ancient gene transfers might have been mediated by large herpes-like viruses with retrotransposons incorporated in their genomes (49). This latter mode of gene transmission might have been carried out by an ancestor of the EBV, inasmuch RAG-like and V(D)J-like genomic segments are still present in the extant descendant of that virus (114). Such as, Marek's herpesvirus harbors the genome of a reticuloendothelial retrovirus (79).

Laboratory-designed genetically engineered viral vectors imitate nature by inserting genes into the tumor-bearing host for tumor suppression, apoptosis induction, immunostimulation by cytokines, and/or for encoding antigens to be recognized by immune lymphocytes and natural killer cells, or by adoptively donated immune lymphocytes.

Genes of interspecies origin in multicellular organisms. The biosynthesis of certain aromatic compounds follows the shikimic acid (shikimate) pathway (115,116). The shikimic acid pathway in the sea anemone *Nematostella vectensis* is encoded by genes acquired through HGT from bacterial and eukaryotic (dinoflagellate) donors (115,116). Tenacibaculum species (*T. aiptasiae, T. adriaticum*) infect anemones and bryozoans (117,118) and tenacibaculum-like gene orthologs, including 16S rRNA of *Flavobacteriaceae* origin, are present in the anemones' genomes (116).

The intracellular bacteria/rickettsiales, Wolbachia sp (*W. pipientis*), have taken up endosymbiotic relationship with insects from termites to mosquitoes, drosophila, wasps and some nematodes. The entire wolbachia genome may be incorporated into one of the insect's chromosomes, thus becoming a heritable endosymbiont. The wolbachia genome is loaded with insertion sequences (IS), the prokaryotic autonomous transposable elements encoding transposases (119-127). Wolbachia strains horizontally transfer among each other their IS genes. Mitochondrial-nuclear discordance in insect cells favoring the spread of carrier insects was observed in Texas (123). The mosquito gene encoding malaria sporozoite receptors in the salivary gland of the insects was picked up by the wolbachia genome (127). Most of the wolbachia genes transferred to the insect's genome become non-functional. The ORF3 (open reading frame) of the drosophila retrotransposon/retrovirus gypsy (see above) encodes the Env protein. The genome of *W. pipientis* acquires the *env* gene from the drosophila genome by horizontal transfer of the entire gypsy retrotransposon (71). The biological consequences of this ancient endosymbiosis have not as yet been defined.

The mollusk *Elysia chlorotica* feeds on the alga *Vaucheria litoria*. While the algal cells are digested, the algal chloroplasts remain functional in the mollusk. This is possible because a reverse transcriptase-containing virus in the mollusk transfers the algal gene for the plastid manganese-stabilizing protein from the algal cells into the nuclei and/or mitochondra of the mollusk's intestinal tract. Thus the virally vectored algal gene continues to encode in its new host the proteins necessary for the function of the algal plastids (128,129).

3. Fused oncogenes in human sarcomagenesis and a hidden retroviral pathogen

Sarcomagenic retroviruses. After the retrotransposon/chromoviral invasion of the genomes of cartilaginous fish (*Chondrichthyes*) leading to the installment of the entire adaptive immune system (see above), teleost bony fish had to sustain the emergence of oncogenic retroviruses. The combined innate and adaptive immune systems surrendered to retrovirally induced sarcomagenesis; these retrovirally induced tumors persist up to the present. However, in their natural hosts, regressions and relapses of these tumors seasonally alternate. The salmon swim bladder leiomyo-sarcoma virus (SSSV) may be ancestral to murine leukemia retroviruses. However, in the sea, SSSV is a piscine epsilon retrovirus. Its primer binding sites in the host cell genome are methionine tRNA. In the zebrafish exists a related apathogenic endogenous retrovirus (ZFERV). Further sarcomagenic piscine epsilon retroviruses are the walleye dermal sarcoma virus (WDSV) and the walleye epidermal hyperplasia viruses (WEHV) type I and II (113,130-133). The SSSV Env protein shows 39% amino acid sequence homology to ZFERV, and more distant (21% homology) to MPMV (Mason-Pfizer

monkey virus) (see below). The regression of WDSV-induced sarcomas is the result of the production of the ORF C protein by the viral genome. The protein targets the mitochondria and induces intrinsic apoptosis of tumor cells with phosphatidylserine excretion (130). The retroviral cyclin protein of WDSV exerts positive or negative regulation via TATA-binding protein associated factor 9 (seven bases TATAAAA, TATA box; TAF9) on NFκB (nuclear factor kappa B); if NFκB is not phosphorylated and does not enter the nucleus, its response elements are not activated (130,133). Thus this sarcomagenic retrovirus controls its replication and its tumorigenesis.

Sarcomagenic retroviruses abound in nature. Avian, murine, feline and simian (woolly monkey) sarcomagenic retroviruses have recently been condensed in a table (134).

Human sarcoma pathogenesis. Retroviral particles were sighted in human sarcoma cells (49), but no human sarcomagenic retrovirus has as yet been isolated and identified. The existence of human sarcomagenic retrovirus(es) is supported by antigenic conversion and focus formation cell-free human sarcoma fluid extracts induce in human embryonic cells (49,134,135). Occasionally retroviral particles appear in human sarcomas and sera and lymphocytes of patients with sarcomas react with autologous and allogeneic sarcoma cells (49,136). These phenomena are documented in microphotographs in the monograph (49) and in other publications (134,135). In Kaposi sarcoma (KS), it appeared as if the patients' antibodies reacted with the budding endogenous retroviral particles (49,134). In the monograph (49), several figures show both herpesviral (displaying the morphological features of the much later discovered HHV-8, the KS-associated herpesvirus, KSHV) and budding retroviral particles (morphologically and immunohistochemically different from the human immunodeficiency virus, HIV-1). In the paper (49) figure 4.18AB shows the budding out of the cell membrane of KS cells endogenous retroviral particles and reaction of the patient's serum in indirect immunofluorescence assay with these virus particles.

Fusion oncogenes and oncoproteins are induced in most of the human sarcoma cells. It is presumed that siRNAs, APOBEC proteins and TRIM motifs (apolipoprotein B-editing enzyme catalytic; tripartite coil-coil proteins) interfere with the isolation of the human sarcoma retrovirus(es) (49,135). Soft tissue (rhabdomyosarcoma) or osteogenic (Ewing's) sarcomas originate from mesenchymal stem cells with mutated and fused oncogenes (137,138), but the causes of the mutations and the inducers of the gene fusions are not known. The elusive human sarcoma retrovirus is the suspect culprit (49).

In KS cells, co-existence of HHV-8 and an endogenous retrovirus (49,134,136) suggests that HHV-8 activates the full maturation of the retrovirus (since HHV-8 activates the formation of retrovirus particles in pleural effusion lymphoma cells) (88). In the monograph (49), Figure 11.4C shows the picture of a disintegrating KS cell taken in 1974 in the pre-AIDS (acquired immunodeficiency syndrome) era. The figure shows the co-existence of retroviral and herpesviral particles. Immature herpesviral particles pack the cell nucleus, and fully mature herpesviral particles are visible in the extracellular space. Budding retroviral particles appear in the cytoplasmic debris; these retroviral particles display morphological and immunohistochemical features very much different from those of the much later discovered HIV-1 particles.

Superinfecting human sarcoma cells *in vitro* with EBV or HHV-8 may activate the hidden human sarcoma retrovirus. In pediatric leiomyosarcomas EBV prevails (49); is there an activated retrovirus in the background? Retrovirally mediated cell fusions might have occurred in multinucleated cells of inflammatory malignant fibrous histiocytomas (139-141). The isolation and identification of human sarcoma retrovirus(es) will be a major breakthrough in retroviral oncology and immunology (49,142,143).

4. Malignant cell fusions

Epithelial to mesenchymal transition. Malignant cells subvert the cell populations that are forming their microenvironment. Regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), vascular endothelial cells, fibroblasts (myofibroblats) and macrophages readily yield to the tumor cells in providing protection against immune T and natural killer (NK) cells (49), and are secreting substances (growth factors and chemokines) to promote neoangiogenesis (vascular endothelial growth factor, VEGF), tumor cell divisions and locomotion. Tumor cells exhibiting mesodermal traits are suspects of hybridization with healthy, but subverted cells of mesenchymal derivation. The genes that control the development of the mesoderm in the embryo are activated in these tumor cells. These are the *snail/slug/smuc* (snail-related skeletal muscle cells) and *twist (twi)* genes. These genes were originally discovered in the drosophila, but they are widely distributed in primitive multicellular organisms (amphioxus, *Branchiostoma floridae*; Cnidarians, the anemone *Nematostella vectensis*; the coral, *Acropora millepora*; and the jellyfish, *Podocoryne carnea*), the lamprey (*Petromyzon marinus*) and remain conserved throughout evolution in vertebrates (frogs, *Xenopus*; zebrafish, *Dario rerio*; birds, *Gallus gallus*) up to the mammals (*Mus*), including *Homo* (144-148).

In the cell metabolism, Snail proteins are repressors of E-cadherin transcription and expression, and thus contribute to the phenotypic epithelial to mesenchymal (ETM) transition of cells. The *snail* family gene product proteins act as cellular survival factors and promoters of cell locomotion. In malignantly transformed cells, they act as supporters of oncogenes (149). A Snail protein may bind and suppress p53 (an anti-apoptotic act in a malignantly transformed cell). However, another oncogenic protein, K-Ras (Kirsten rat sarcoma), can antagonize Snail-p53 binding and thus preserve p53 expression and function (150). On the other hand, it is the Ras oncogenic proteins that activate the TGF-ß (transforming growth factor-beta) genes to produce the cytokine; in turn, TGF-ß activates Snail protein production, thus initiating the process of ETM transition; lymphoid enhancer factor-1 (LEF-1 encoded from 4q23-q25) is essential for the interaction (151,152). The transcription factor cyclin D is inhibitory to the cell cycle (mitoses) in melanoma cells. The Snail 1 protein starts a cascade by inhibiting cyclin D in melanoma cells (153). Consequentially, the proto-oncogene protein Bcl-3 (B cell lymphoma) translocates into the nucleus to

activate the genes responsible for melanoma cell proliferation and invasion (N-cadherin promoters). In melanoma cells, ets/YY (*ets* oncogene of avian erythroblastosis leukemia retrovirus E26; yin yang) regulates snail gene transcription (154). Snail interacts with ß-catenin at its N terminus and thus activates it. Up-regulation of the Wnt/ß-catenin (wingless in drosophila; *int* in mice) pathway by the Snail protein in colorectal and breast cancer cells increases the cells' malignancy. The WNT-1-induced signaling protein-3 (WISP3) exerts tumor suppressive effect in inflammatory breast cancers. When WISP3 is inhibited, E-cadherin expression is reduced and the tumor cells invade and metastasize. In response, the E-cadherin transcriptional repressor Snail becomes up-regulated. In cells with Snail blocked by siRNA, WISP3 inhibition failed to down-regulate E-cadherin (155,156). Thus the Snail protein interacts with WISP3 and by inducing ETM transition it promotes the expression of the malignant phenotype. The Snail protein is transported into the nucleus by importin-ß (157). The p70 S6 kinase activates the snail gene in ovarian cancer cells and thus induces ETM transition with increased malignant potential (158). The regulators of orderly embryogenesis, the Twist proteins, are the major promoters of the epithelial-mesenchymal transition (EMT) in malignant cells, where they antagonize p53 and Rb and collaborate with Ras. In melanoma cells, Twists suppress the interferon pathway (159) (see below), thus depriving the host of a possible natural defense against this particular malignancy (but may be promoting susceptibility to oncolytic viral therapy, inasmuch as the oncolytic virus will not be subdued by interferon production within the tumor cell). The locus of the human snail 1 gene was mapped to 20q13.1-2 (160,161). The protein product contains 264 amino acids; the carboxy-terminal is the very active zink finger DNA-binding domain (157).

The locus of the *sparc* (osteonectin) gene in the human genome is at 5q31.3-q32 and its gene product 32-kDa matricellular glycoprotein is SPARC (secreted protein acidic, rich in cystein). SPARC is another suppressor of E-cadherin and inducer of ETM transition. SPARC induces the nuclear translocation of ß-catenin, thus promoting the interaction of ß-catenin with T cell lymphoid enhancer factor-1 (162). By its activation of ß-catenin signaling, SPARC is anti-adipogenic (163) (see below). The fibronectin matrix assembly is SPARC-dependent (164). The up-regulated Twist, Snail and Slug proteins (165,166) together in malignantly transformed human cells repress the vascular endothelial VE-cadherin promoter (166).

SPARC-over-expressing melanoma cells lose their adhesion to vitronectin (but not to fibronectin) and assume mesenchymal cell phenotypic features with increased malignancy, which is accelerated locomotion and metastasis formation (167). The CCN3/NOV proteins regulate melanocyte proliferation and their adherence to the basement membrane (CCN3/CYR3, cystein-rich angiogenic protein encoded from 1p22p31; NOV, nephroblatoma overexpressed gene at 8q24.1). The CCN nomenclature is published by the International CCN Society: http://ccnsociety.com; CCN 4, 5, 6 = WISP 1, 2, 3 (see above) (168,169). IL-1ß is a CCN3 inducer; it could suppress the growth of low-grade melanoma cells. High-grade melanoma cells down-regulate CCN3 expression, do not respond to IL-1ß and up-regulate SPARC (170). SPARC over-expression is especially dominant in melanoma cell-macrophage hybrids. Macrophage-derived SPARC promotes migration of tumor cells (171). SPARC effects are mediated by leukotriens, interleukin IL-8 and Fas ligand (L) expression resulting in the removal of polymorphonuclear leukocytes, which would otherwise react to FasL-expressor melanoma cells (172). Antisense-mediated down-regulation of SPARC in melanoma cells resulted in the expression of decreased levels of N-cadherin and clusterin and increased levels of HSP27 (heat shock protein 27 kDa encoded from 7q11.23) (173). Genetic engineering-produced or purified native SPARC proteins are available for experimentation with tumor (melanoma) cells (174). Down-regulation of SPARC in malignant glioma cells results in decreased cell migration and invasion (175). While SPARC inhibited the ovarian cancer cell's integrin-mediated adhesion to the matrix, it also inhibited the stimulation of the AKT- and related cell survival pathways (AKT derives from a murine thymic retroviral v-*akt* oncogene and its c-*akt* counterpart) by epidermal growth factor (EGF) (176). Thus, SPARC may exert anti-proliferative and proapoptotic effects (176). It is integrin $\alpha_v ß_5$ over-expression that macrophage-derived SPARC promotes (171); the related integrin $\alpha_v ß_3$ is the vitronectin receptor.

The consequences of SPARC over-expression in malignant cells is not uniformly that of increased locomotion and metastasis formation (see above). In some adenocarcinomas (colorectal, ovarian), highly SPARC-positive cancer cells may be suppressed rather than over-stimulated (177). Indeed, in embryonic life, SPARC promotes endoderm differentiation and cardiomyogenesis in generating beating-pulsating cardiomyocytes. For these studies a recombinant baculovirus-produced SPARC protein is available (178). Another physiologic function of SPARC is inhibition of adipogenesis and altering the composition of the extracellular matrix. SPARC inhibits the differentiation of pre-adipogenic stem cells (stromal-vascular cells) into fusiform pre-adipocytes and the transformation of these into sphere-shaped adipocytes. These round adipocytes alter the stroma to be laminin-rich and fibronectin-poor. SPARC counteracts by promoting the formation of a fibronectin-rich stroma (163). In the case of melanoma cell-macrophage hybrids (see below), the melanocortin-induced chemotactic phenotype with the integrin fibronectin receptor $\alpha_5 ß_1$ expression, is promoted. These cells are attracted toward fibronectin-rich pathways for locomotion (163). SPARC induces the nuclear translocation of ß-catenin, thus inducing interaction between ß-catenin and T cell/lymphoid enhancer factor 1 (162,163). Twist is activated in macrophages producing inflammatory cytokines (see above). When a melanoma cell secretes inflammatory cytokines, it is a suspect to be a macrophage hybrid, therefore over-expressing the *twist (twi)* gene (159). The *twist* gene-inducer, milk fat globule, MFG-E8, secreted by high-grade melanoma cells promotes ETM transition and vertical growth phase (179). Tumor cells increase their malignant potential either by up-regulating their own *snail-slug-smuc-sparc-twist* genes, or by fusing with a mesenchymal cell willing to provide these genes in an active state. The last paragraph of Discussion describes the acquisition of carcinogenic potency by human breast cancer stem cells generated during the process of ETM transition.

Lymphoma cells fused with plasma cells or with Treg cells. In EBV$^+$ nasopharyngeal carcinoma cells the latent membrane protein (EBV-LMP$^+$) activates *twist* (180). The *twist* gene can be activated by NFκB (nuclear factor kappa B) when NFκB translocates from the cytoplasm to he nucleus. The re-arranged cytoskeleton allows the accelerated amoeboid locomotion of the tumor cells. Theories were proposed for the origin of some Reed-Sternberg (RS) cells by cell fusions, as if the progenitor cell harboring a retrovirus had fused with an EBV$^+$ B cell (181). However, the German school marshaled strong arguments for the single B cell origin of most of the RS cells (182). In this view, it remains unexplained how multinucleation occurs in RS cells and why would one nucleus show p53 mutation, while the other nuclei of the same cell would be exempt from this mutation (183).

For the formation of Sézary cells (the flower cells; clover-leaf cells), this author theorized that the malignant diploid CD4 (cluster of differentiation) helper T cell of mycosis fungoides (MF) engulfed an autologous Treg cell for its own protection from FasL$^+$ immune T cell attack by the host. By expressing chemokine receptor CXCR, MF cells may attract Treg cells which express the chemokine ligand. The tetra/polyploid Sézary cell is armed with up-regulated FoxP3 pathway, anti-apoptotic Bcl-2 and c-Myc (avian myelocytosis retroviral-oncogene); and auto-, or paracrine circuits for IL-7 and IL-15 (49). Since Zucker-Franklin's work (184), it has been repeatedly documented that HTLV (human T cell lymphotropic virus)-like retrovirus particles are expressed in MF and Sézary cells. Are these virus particles mediators of the fusion between MF cells and Treg cells?

This author in the mid-1960s observed spontaneous *in vivo* fusion of diploid B lineage mouse lymphoma cells with healthy B (plasma) cells of the host. The lymphoma cells produced budding retroviral particles of the Rauscher mouse leukemia virus complex, a mixture of an erythroleukemia (Friend-like) and lymphatic leukemia (Gross-like) virus, and recombinants thereof (185,186-190). The healthy plasma cells involved in the fusion process secreted specific antibodies neutralizing the leukemia virus. The fused cells (cell line #818) could be grown in suspension cultures for over 10 years and continued to secrete the specific antibody. Immortality of the cell line was endowed by the retroviral genomes. The #818 cell line gained unprecedented virulence *in vivo*. While the diploid lymphoma cell line (cell line #620) grew slowly in the form of solid tumors, and its small incipient tumors could be rejected by the hosts, the tetra/polyploid #818 cell line, the result of fusions between #620 cells and antibody-secreting immune plasma cells, rapidly disseminated in the entire body of the inoculated mice and grew as large hemorrhagic ascitic tumors. Tumors of #818 cells never could be rejected. However, the histological picture of #818 tumors was characterized by an extensive appearance of the 'starry sky' phenomenon. The starry sky phenomenon consisted of disintegrating lymphoma cells within macrophages. The explanation was offered that the antibody-coated #818 cells could not be recognized by immune T cells (due to the coverage of budding retroviral antigens by self antibodies). However, the antibody-coated #818 cells activated FcR-positive macrophages inducing their engulfment and digestion by the macrophages. Most of the macrophages of the starry sky phenomenon showed 'nuclear clumping' and died (not recognized in the 1960s as apoptosis).

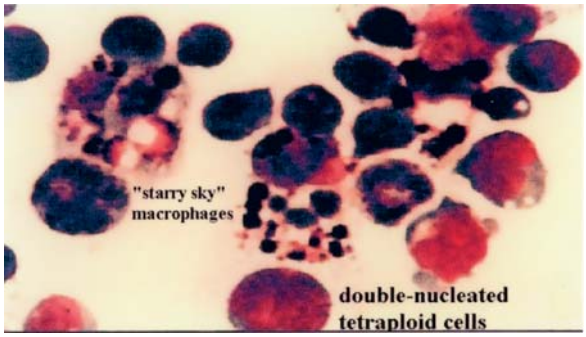

Figure 1. Murine leukemogenic retrovirus- the Rauscher virus complex - producer murine lymphoma cells fuse with antibody-producer immune plasma cells of the same mouse strain in the peritoneal cavity of mice. The mice were inoculated with the mixture of retrovirus-producer #620 diploid lymphoma cells and immune spleen (plasma) cells taken from mice actively immunized with a photodynamically inactivated leukemia virus vaccine (49,186,187,189,190,360). This was the first exmple of 'natural hybridoma' formation occurring in the late 1960s (192). Large macrophages phagocytose antibody-coated fused lymphoma cells. This event is a reproduction of the 'starry sky' phenomenon occurring during the course of the #620 lymphoma *in vivo* (361). Both lymphoma cells and macrophages die in this encounter. Cell death with 'nuclear clumping' was not recognized as due to apoptosis in the mid-1960s. The cell lines #620 and #818 were submitted to Trujillo *et al* for further studies (362) and for guardianship of the cell lines in storage deep frozen. These cell lines were lost during the flood of the Texas Medical Center consequentially to hurricane Allison in June 2001 and due to the death of their guardian, Dr Jose Trujillo. Published with permission from ref. 190 and vol. 49 (j.schenk@schenkbuchverlag.de).

The spontaneous fusion of #620 diploid lymphoma cells with antibody-secreting plasma cells was reproduced. Diploid #620 lymphoma cells were mixed with plasma cells obtained from the spleens of mice vaccinated against the leukemia virus. A photodynamically inactivated mouse leukemia virus was used to immunize these spleen cell donor mice (Fig. 1). The plasma cells produced leukemia virus neutralizing antibodies. The cell mixtures were injected intraperitoneally into young adult Swiss mice. In the peritoneal cavities fusion of the diploid lymphoma cells with immune plasma cells was directly observed. Further, peritoneal macrophages phagocytosed the fused cells, thus reproducing the 'starry sky' phenomenon (Fig. 1). Some 6-8 years later, after the publication of artificial hybridoma production in the laboratory by Köhler and Milstein (191), the phenomenon observed some 6 years earlier in mice was now referred to as 'natural hybridoma' formation (192). Recognition of this phenomenon was reported first in the Lancet in 1970 with these words written in 1969 (186): 'Tetraploid immunoresistant lymphoma cells in the mouse emerge by fusion of the diploid virus-producing lymphoma cell with a plasma cell producing virus-specific globulins. The resulting tetraploid cell will retain malignant growth potential and the genetically determined committedness of both parent cells to produce leukemia virus, as coded for by the viral genome within the neoplastic cell, and to synthesize virus-specific globulins, as coded for by the genome of the plasma cell'.

This author working with J.M. Trujillo at M.D. Anderson Hospital in the mid-1960s and late 1970s isolated lymphoma cell lines from a patient (Trujillo's T1; Sinkovics' #778 cell

lines from patient HF MDAH#54537) (190,193-195), in which lymphoma cells replicated an unidentified retrovirus and coexisted with plasma cells producing immune globulins. Lymphoma cells fused with lymphoma cells and with plasma cells formed large cell conglomerates (Fig. 2). Actual fusions of lymphoma cells with autologous plasma cells were photographed (49,190,193). However, the #778 lymphoma cells single or fused were not attacked by monocytes-macrophages (there was no attraction exhibited by the #778 lymphoma cells to FcR^+ macrophages). Accordingly, in the histological pictures of the lymphoma, no 'stary sky' phenomena were observed. However, *in vitro* #778 lymphoma cells single or fused were attacked by small compact lymphocytes recognized to be immune $CD8^+$ T cells (these cells are devoid of FcR). These events suggest that the cytoplasmic retroviral particles were not coated by antibodies inducers of an FcR-mediated reaction. Thus the cell fusions might have been induced by a fusogenic retrovirus, rather than a retroviral antigen (in the lymphoma cells) and a specific antibody (in the plasma cells) reacting with each other. Thus, lymphoma cell to lymphoma cell fusion events might have dominated over lymphoma cell plasma cell fuions. Nevertheless, this author proposed that 'natural hybridoma formation' occurs in certain human lymphomas (49).

Hybridization of mouse B lineage splenic cells with plasmacytoma cells resulted in the generation of metastasizing tumors of distinct organ specificities: metastases in both liver and spleen, or in either liver or in spleen (196).

Tumor cell fusion partner monocytes-macrophages. Allogeneic bone marrow recipients later developed renal cancers, which metastasized. The donors were free and remained free of renal cancers. In the tumor cells, the blood group antigen of the donor, or the Y chromosome of the donor in a female recipient (mother and son) were recognized (197,198). Thus the metastasizing tumor cell fused with some of the allogeneic donor bone marrow cells (199).

The most common healthy cells fusing with tumor cells are the monocytes-macrophages. Munzarová and Kovarík proposed that melanoma cells fuse with host macrophages and thus gain growth advantages (200). A patient with relentlessly advancing metastatic melanoma resisted chemotherapy, vaccinations combined with cytokines: interferon-α, interleukin-2, and GM-CSF (IFNα; IL-2; granulocyte-macrophage colony stimulating factor, Leukine, Roche), oncolytic viral therapy, and adoptive immune lymphocyte therapy with IL-2 (201). His large and mobile melanoma cells phagocytosed red blood cells; it was presumed (but not proven) that his melanoma cells hybridized with macrophages.

When melanoma cells express myeloid-macrophage-related antigens ß,1,6-branched oligosaccharide N-glycans, GnT-V (N-acetylglucosaminyltransferase V) focal adhesion kinase, neurotrophin and its receptor for autocrine circuits, Toll-like receptor-4 (TLR-4) and thus respond to lipopolysaccharides, undergo vascular mimicry formation, or express multidrug resistance efflux pump proteins, or enter the state of autophagy, they are suspect of hybridization with host macrophages. The lysosomal-associated membrane proteins (LAMP) are substrates for GnT-V. Overexpression of LAMPs in metastatic melanoma cells (melanoma-macrophage hybrid

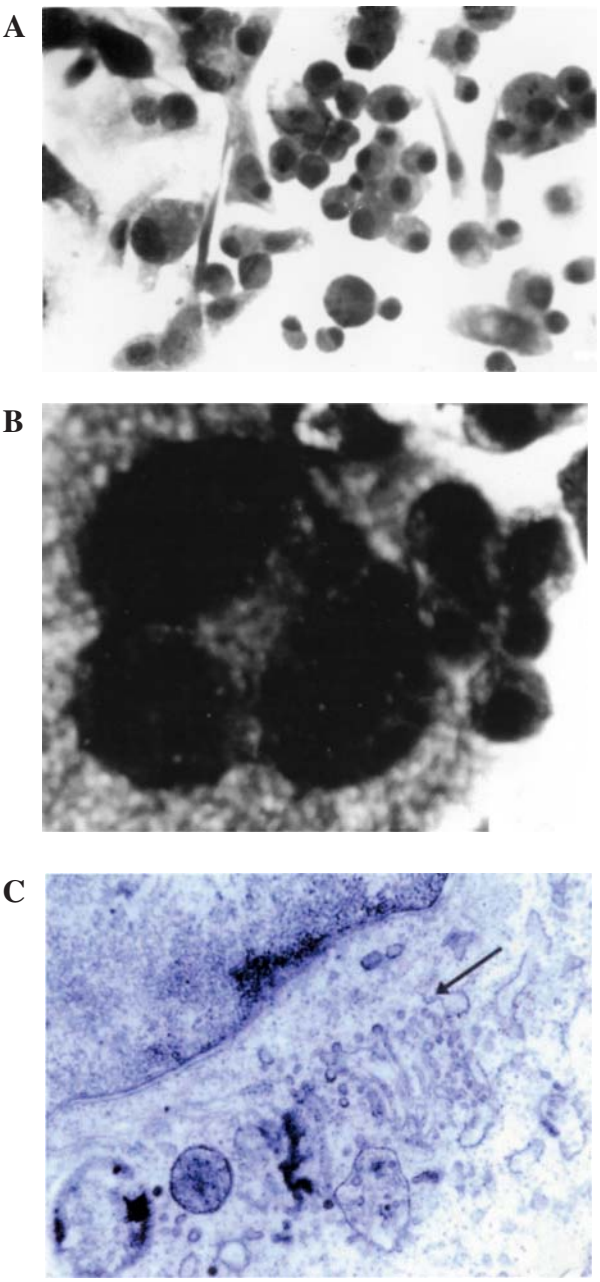

Figure 2. (A) Lymphoma cells of human lymphoma cell line #778 fused with plasma cells of the patient in an *in vitro* primary culture. (B) Large conglomerate of fused lymphoma cells in culture #778. The large fused cells induce the attachment of reactive autologous small compact lymphocytes (immune T cells) (190,193). (C) Cultured #778 cells express retroviral particles budding within the cytoplasm (49,190). The budding envelopes encircled the distance of about 120 millimicrons. Presumably the retroviral antigens in the lymphoma cells attracted antibody-produer plasma cells, which fused with the lymphoma cells: a 'natural hybridoma' formation in a human lymphoma. The antibody-coated lymphoma cells did not visibly attract NK cells or macrophages (there was no 'starry sky' phenomenon in the histological picture). Therefore the cell fusions might have occurred due to another mechanism, that is, if the retroviral agent was fusogenic and lymphoma cells fused with lymphoma cells. Lymphoma cells of the same patient were cultured by Trujillo *et al* (194,195). Retroviral production in those lymphoma cell cultures was also observed (195). B lineage cells in the culture secreted immunoglobulins; presumably the sources of the immunoglobulins were either the lymphoma cells, or the plasma cells, or both. Trujillo *et al* have not considered 'natural hybridoma formation' in their cultures. These cell lines were lost in the Houston, TX, Medical Center flood following Hurricane Allison in June 2001 and consequentially to lack of emergency rescue efforts due to the death of their guardian, Dr Jose Trujillo. Published with permission from ref. 190 and vol. 49 (www.buchverlag-schenk.de).

cells) promotes adhesion of melanoma cells to vascular endothelial cells (202-204) (for whatever advantages the melanoma cells could thus exact). Interestingly, GM-CSF is included in certain melanoma treatment protocols as a stimulator of DCs. It may increase response rates and delay relapses (205,206), but these additional benefits are not unequivocally confirmed (207). Single melanoma cells may be killed by a host immune attack thus induced, while subclones of melanoma cell-macrophage hybrids may be stimulated by GM-CSF and cause a relapse. Some melanoma cells express IL-2R and undergo divisions upon IL-2 administration (208). Are these melanoma cells fused with host T cells?

Some melanoma cells re-unite broken chromosomes 1 and 10: t(1;10)(p32-34;q23-26). If this happens, stimulation of the extracellular domain of the Fas receptor (cluster of differentiation, CD95), the gene of which is mapped to 10q23-26, with its ligand (L), FasL, takes place. Signaling runs downward from CD95 to the misaligned G-CSF receptor's (granulocyte colony stimulating factor) intracellular domain (encoded from locus 1p32-34). Such a misdirected signal transduction induces a cell division. Thus a melanoma cell could use the death domain of the FasL-to-FasR system for its own growth stimulation (209-211). The circuit could be an autocrine one, in which the Fas receptor-positive melanoma cell produces the ligand FasL. The Fas and G-CSF receptor (R) proteins could be fused like that *in vitro* (212). Melanoma cell-derived FasL kills $FasR^+$ host lymphocytes, but FasL-expressor tumor cells attract leukocytes. (reviewed in ref. 49). The reactive leukocytes either attack the melanoma cells or fuse with them. Glioblastoma cells could use the FasL to induce their own mitoses in an autocrine circuit (213). In reviewing apoptotic and anti-apoptotic forces in neoplasia, a melanoma cell line was presented in which FasL (purified by J.C. Horvath) induced mitoses that were inhibited by an anti-FasL monoclonal antibody (210). *Errata*: the artist mistakenly showed as if the CD117 c-Kit receptor would capture as its ligands the hepatocyte growh factor/scatter factor. In truth, these ligands act with receptor c-Met (see below). The c-Kit receptor's cognate ligand is stem cell factor (210). Due to type-setter's mistake, the Fas receptor written in the manuscript as CD95 was printed as CD9S (209).

Mesenchymal gene expressions in melanoma cells may be, or may not necessarily be, the result of fusion of the melanoma cell with a myelocyte or leukocyte. The native melanoma cell may activate its own G-CSF/GM-CSF genes to perform for its own growth advantage.

Fusogenic viruses. Malignant murine T lymphoma cells gained virulence after fusion with host macrophages (214). Methylcholanthrene-induced murine sarcoma cells fused spontaneously with host macrophages *in vivo* and thus gained increased neovascularity and growth rate (215). The fusion was termed 'spontaneous', because no search was done for a fusogenic virus. Fusogenic retroviruses abound in nature and frequent tumors. In murine species, these viruses are related to the mouse leukemia retrovirus M813 isolate from *Mus cervicolor* (216), and in simian hosts, to the Mason-Pfizer (MP) retrovirus (217,218). The MP monkey virus (MPMV) fuses human cells, induces aneuploidy and eventually malignant transformation in the hybridized cells (218). These native viruses induce, or promote, the fusions of malignantly transformed human cells. In this case, tumor cells operating different oncogenic pathways may unite these in one polyploid tumor cell of increased malignancy (219). The MPMV actually causes cancer in fused cell by inducing chromosomal instability, meaning that fusogenic virus-containing exosomes are generated and by inactivating of tumor suppressor protein p53, remove the brake cell fusion poses against proliferation (220).

Viruses inducing tumor cell syncytia may be protected from host antibodies, inasmuch as the viral particles spread from cell to cell intracellularly within the tumor cell syncytia. If the virus is oncolytic (vesicular stomatitis virus, VSV) and eventually lyses the tumor cell syncytia (49,221), it is to the advantage of the host that the virus was not prematurely neutralized by the antibodies.

Chimerism of tumor cells and normal host cells, especially macrophages usually results in increased malignant potential of the tumor cells. For example, melanoma cells fused with macrophages gain virulence (202-204). However, many human melanoma cell clones express fully mature (budding out of the cell surface) endogenous retroviruses (222), which are implicated in the malignant transformation of the nevus melanocytes (86). It is an attractive presumption that these endogenous retroviruses induce the fusion of melanoma cell-macrophage hybrids. The proposal of such an event is quite plausible. The human genome harbors at least 18 retroviral coding envelope (*env*) gene sequences. Some of these genes were inserted over 40 million years ago, whereas some others were inserted less than 5 million years ago. This last date is set after the divergence of the *Pan* and *Homo* lineages. The major contributor to the most recent *env* gene insertions is the HERV-K family of human endogenous retroviruses (see above, and Syncytins below). The *env* genes with a full length ORF are fusogenic to cells attached to one another (223). This event, while contributing to the virulence of the tumor cells, may be stimulatory to the host to break tolerance and react to the retroviral envelope proteins with antibody production and NKT cell activation (224), or with immune T cell mobilization, as in tumor cell xenogenization (224). In contrast, some retroviral envelope proteins expressed by tumor cells exert immunosuppression in the host (225).

Macrophages and DCs phagocytose apoptotic or necrotic tumor cells. Necrotic tumor cells may be more immunogenic than apoptotic tumor cells (49) (see below). Would DNA segments of the apoptotic cell function in a genetic or epigenetic manner in its new host, the macrophage? Indeed, EBV genomic segments (EBER, EBNA, Epstein-Barr virus RNA transcription unit; EBV nuclear antigen) deriving from phagocytosed apoptotic lymphoid cells appeared integrated in the recipient cells' genomes (226). Certainly, preservation of EBV genomic segments in memory B cells, resting and proliferating B and T lymphoid cells, and in malignantly transformed cells (B and T lymphoid cells; epithelial cells of the nasopharynx and stomach; smooth muscle cells in childhood leiomyosarcomas) are of common occurrence (49). Macrophages are inclined to revert to autophagic existence under adverse circumstances. Thus metastatic melanoma cells expressing autophagic genes and thus autophagosomes, might have acquired this propensity from a macrophage with

which the melanoma cell fused (202-204). However, virally infected and not killed cells often linger in the autophagic state. Oncolytic adenoviral vectors induce autophagy in malignant glioblastoma cells (227,228). Autophagic tumor cells are not necessarily so due to fusion with a macrophage (204-206): autophagy may be a sign of viral infection, attack by cytotoxic lymphocytes (49), or a survival strategy of a chemotherapy-damaged tumor cell (229).

MET, MC/MSH-R, MITF. The *met* proto-oncogene is active in normal embryogenesis (230). It acts as an oncogene in the N-methyl-N'-nitro-N-nitrosoguanidine-(MNNG)-transformed human osteosarcoma (HOS) cell line (231-233). The MET oncoprotein is the receptor for hepatocyte growth factor/scatter factor (HGF/SF) ligands (213) (see above). The locus of the *met* proto-oncogene in the human genome is mapped to 7q31.2. Met transformation alters the tumor cells' surface by enhancing the expression of laminin and collagen receptor complexes (the integrins α6/ß1, α2/ß1, α1/ß) (234), thus promoting invasion and may be it is thus contributing to the fusogenicity of the tumor cells.

The two main receptors of melanocortin (MC) peptides are the melanocyte-stimulating hormone receptor at 16q24, and the melanocortin receptors at 18q22/20q13.2 (235). The microphthalmia-associated transcription factor (MITF) regulates pigment cell differentiation including those of the retina. Its 'master gene' is mapped to 3p14.1-2 in the human genome; it exists and operates in many isoforms. Its gene product proteins bind nuclear DNA; in turn, the targeted DNAs encode tyrosinases and tyrosine-related proteins. Further, a MITF protein promotes melanocyte (melanoma cell) proliferation by directly activating the cdk2 (cyclin-dependent kinase) promoter. In contrast, MITF by activating p16Ink4A (cdk inhibitor) and p21Cip1 (cdk-interacting protein) arrests the cell cycle. MITF interacts with oncoprotein B-RAF, either counteracting, or stimulating it. MITF may act in melanocytes as a cell survival oncogene by activating B-RAF and the anti-apoptotic Bcl-2 (of note, the *ras*-related, rat sarcoma, oncogene raf stands for rat fibrosaroma and encodes the ABC RAF oncoproteins). In contrast, a MITF C-terminal product arrived at by cleavage is pro-apoptotic. MITF requires the chromatin remodeling enzymes SWI/SNF (switching, in saccharomyces and *sans fille* in drosophila) in order to activate melanocyte-specific genes (236-239). Overexpression of all the above listed receptors in metastatic melanoma cells strongly suggests that these tumor cells have hybridized with macrophages and are driven by an excessive gene load to grow by capturing their ligands MC, MSH and HGF/SF. These systems work in both paracrine and autocrine circuitries (204-206).

Regaining tumor suppressor gene expression. Loss of tumor suppressor gene function is due to germ cell or somatic cell mutations, or both. Loss of heterogeneity (LOH) and a failure to repair DNA damage are further major contributory factors to oncogenesis. Tumor cells fused with normal fibroblasts or vascular endothelial cells may regain oncosuppressor genes resulting in the loss of oncogenicity of the fused cells. While the congenial prediction of A. Knudson (during his tenure on the staff of M.D. Anderson Hospital, Houston, TX), that retinoblastomas arise due to germ cell and somatic cell mutations (240) lead to the discovery of the human retinoblastoma gene (mapped to 13q14.2) and its gene product protein acting as a brake of the cell cycle. The decreased or annulled malignancy of hybrid (chimeric) malignant and normal cells strongly suggested the existence of, but failed the actual identification of, further human tumor suppressor genes (241). The anti-apoptotic p53 gene emerged as another human tumor suppressor gene (the guardian of the genome) and consequentially this gene is being replaced (re-inserted) by adenoviral vectors into human tumor cells *in vivo*. These adenoviral vectors of the p53 gene are too numerous to list, but their review has been provided (242,243). However, the frequent LOH at chromosome 3p21.3 in human tumor cells was suspected to be the site of another human tumor suppressor gene (241), however, this gene has not as yet been identified (244). Cell fusional events between melanoma cells and macrophages result in increased malignant potential (245), whereas melanoma cells fused with normal fibroblasts were deprived of their malignancy (246,247), as reviewed extensively (199,204-206). Polyethylene glycol or the hemagglutinating virus of Japan (HVJ), the Sendai virus, exert these cell fusions. The genome-depleted HVJ envelope remains fusogenic (HVJ-E@iskweb.co.jp). The monochromosome transfer technology identified the cyclin-dependent kinase inhibitor gene cdkN2A/16INK4A mapped to 9p21), as a most powerful tumor suppressor (248).

Osteoclast fusions. Multinucleasted osteoclasts arise through physiological cell fusions mediated by the macrophage fusion receptor (MFR) interacting with the thrombospondin 2 receptor, and signal regulatory protein alpha (SIRGA). Thrombospondin 2 receptor is recognized as CD47. The chemokine, dendritic cell-specific transmembrane protein (DC-STAMP), and its presumptive chemokine ligand, CCL2, the monocyte chemoattractant protein 1 (MCP1), are known physiological inducers of macrophage fusions (osteoclasts; giant cell formations in granulomas) (249). However, in multiple myeloma, among the multinuclei of osteolytic osteoclasts there are myeloma cell nuclei (250). Are the myeloma cell nuclei incorporated 'by accident' or are they contributory to accelerated osteolysis by myeloma-associated osteoclasts? Osteoclasts and myeloma plasma cells mutually stimulate each other. Factors deriving from osteoclasts stimulate MAPK, STAT3, and PI3K/Akt cell survival pathways in myeloma cells (mitogen activated protein kinase; signal transducer and activator transcription, phosphatidylinositol kinase; v-*akt* Rockefeller Institute's AKR-derived thymic mouse leukemia AKT8 retroviral oncogene and its c-*akt* cellular oncogene counterpart and Akt oncoproteins, see above). Myeloma cells activate the p38 MAPK, NFκB, MMP (matrix metalloproteinase), cathepsin K, urokinase plasminogen activator and TRAP (tartrate-resistant acid phosphatase) pathways (251). Osteolysis by osteoclasts could be inhibited by the peroxisome proliferators-activated receptor gamma (PPARγ) agonist KR62776 (252). The agonist suppressed TRAP, DC-STAMP, osteoclast-associated receptor (OSCAR), and RANKL (receptor activator of NFκB ligand) (253,254), thus p38 MAPK, NFκB and extracellular regulated kinases (ERK) of osteoclasts all were inhibited (254).

Resveratrol (trans-3,45-trihydroxystilbene) inhibited RANKL, NFκB, and TRAP in osteoclasts and up-regulated osteoblast markers c-*fms*, osteocalcin, osteopontin and vitamin D3 nuclear receptor in bone marrow mesenchymal stem cells (abbreviations: v-*fms*, feline McDonough sarcoma viral proto-oncogene; cellular counterpart c-*fms* encodes colony stimulating factor-1 receptor) (255).

5. Discussion: physiology, pathophysiology, therapeutics

Physiological cell fusions. Mating pairs of *Saccharomyces cerevisiae* fuse; epithelial cell and gametes fuse in the nematode caenorhabditis; myocytes fuse in the salivary gland of the drosophila (256). In the developing *Caenorhabditis elegans*, extensive cell fusions take place. These involve not only fusion of oocyte and sperm, but also of various somatic cells. The gene product protein *eff*-1/EFF is the fusogen. Transcriptional regulatory factors control the activity of the *eff*-1 gene. The product of the *ref*-2 gene, regulator of fusion-2, keeps certain cells unfused, whereas Ras, Wnt, Rb/E2F gene product proteins regulate fusion in the larvae (257) (abbreviations: rat sarcoma genes Harvey and Kirsten discovered in rats inoculated with the Moloney mouse leukemia-sarcoma virus; *wingless* and *int* drosophila and murine gene product proteins; retinoblastoma gene product protein and enhancers of E2 family transcriptional regulators). In the drosophila embryo multinucleated syncytia arise and persist during the entire larval life. The insect's salivary gland epithelial cells migrate to their location only after myoblasts, longitudinal muscle founder cells and other mesenchymal cells fused and provided the proper integrins (258,259). Interspecies hybridization by cell fusion in fishes (carp) resulted in allo-tetraplod hybrid cells of great significance in genetic research for fish breeding (260), Plant (*Solanum*) mesophyll cell protoplasts can be fused *in vitro* with PEG (polyethylene glycol), remain stable as tetra- to decaploid cells, grow in soil, set flowers and bear parthenocarpic (virgin fruit) berries (261-263). This technology is applicable to many fruit-bearing plants.

An early report from Hungary concerned myocyte fusions in the dilator pupillae muscle (264). In heterokaryons formed by muscle cells with non-muscle cells (fibroblasts) resulted in the silencing or elimination of the muscle cell identity gene expressions (265), except for myoD. Cardiac myocytes do not express myoD and when fused with fibroblasts, the heterokaryons fail to express the myocyte phenotype (266). In the normal development of mammalian skeletal muscle, multinucleated myotubes are formed; the cell fusion proteins are those in the classes of metrins, fertilins (the egg-sperm fusion proteins) and metalloproteinases/disintegrins (267). However, not only the nuclei, but also the cytoplasms with the mitochondria are united in fused hybrids. In the ES-splenocyte hybrids, the mitochondria (mtDNA) of the somatic partner were eliminated, whereas ES-fibroblast hybrids retained the mitochondria of both partners (268).

During carcinogenesis, cancer cells and endothelial cells fuse (269). The result may be neoangiogenesis in the form of 'vascular mimicry' (270). The mammalian placenta behaves like a pseudomalignant organ; the growth factors and the immunoevasive maneuvers of malignant tumors and the placenta are quite alike, as shown in a tabulated form in ref. 271. In the Australian marsupial, the bandicoot (*Peramelidae*), fetal trophoblasts fuse with the maternal homokaryons, the highly vascularized columnar uterine epithelium, and so form syncytia (272). During mammalian pregnancy, not only fetal cells enter the maternal blood circulation and vice versa, forming fetomaternal fused entities of 'microchimerism', but also fetomaternal cell fusions take place in the placenta. While these fused cells are eventually evicted with the placenta, fused cells from the systemic circulation settle and persist. These physiological processes may have pathological immunological consequences, such as autoimune disease induction, or contrarywise, tolerance induction (273-275). Recently reported, tumor necrosis factor-alpha (TNF-α) appears in the human placenta and endometrium and may exercise either cell death induction in aggressive trophoblasts, or fusion between fetal and maternal cells. The streptavidin-tagged human TNFα acts as a powerful fusion protein (275a,b).

Syncytins. The cytotrophoblasts of the primate placenta express the retrovirally encoded fusion proteins (envelope proteins) syncytin 1 and 2, fuse, and thus form the barrier of syncytiotrophoblasts (276-280). The fusion protein syncytin-1 is the envelope of the human endogenous retrovirus-W (HERV-W) with its proviral locus at 7q21-22. Syncytin 2 is the product of HERV-FRD with its proviral locus at 6p24.1 (abbreviations: W = HERV-W's primer binding site, PBS, tryptophan-like (W) $tRNA^{Thr}$ of the multiple sclerosis-associated retroviral element (retrovirus), MSRV; F = HERV-F's PBS human phenylalanine $tRNA^{Phe}$; R = HERV-R's PBS mouse arginine $tRNA^{Arg}$; HERV-FRD's PBS histidine tRNAHis; D = mammalian virus receptor) (281-284). In the cytoplasmic tail of syncytin-1, the region adjacent to the transmembrane region is the fusogenic part; the extreme C-terminus of the tail is inhibitory to fusogenicity. Thr syncytin-1 segment truncated after the terminal residues is hyperfusogenic (285). The proviral element of the HERV-FRD gene encoding syncytin-2 (286) was inserted into the simian genome before the divergence of Old World and New World monkeys, inasmuch as it is present in both branches, thus the gene has been conserved over 40 million years (287,288). The human syncytin-2 attaches to the cell surface receptor named major facilitator superfamily domain containing 2 (MFSD2); the receptor's ancient and well conserved gene is mapped to 1p34.2 and it is over-expressed in syncytiotrophoblasts of the placenta and in choriocarcinoma cells (289). Mouse syncytins A and B are distantly related to human syncytins-1 and -2. The human syncytin-2 molecules are fusogenic and protective (immunosuppressive to the host) against rejection of the cells (syncytiotrophoblasts) expressing these molecules (290). While the MSRV induces no immune reactions in patients with MS (291), HERV-K induces immune reactions in those patiens whose breast cancers express these viral antigens (292). Patients with seminoma generate T lymphocytes specifically reacting to HERV-K structural proteins (293). The transformation of placental cytotrophoblasts into syncytiotrophoblasts is often termed 'differentiation', thus HERV Env expression can induce cell differentiation (294).

Cancer cells notoriously expropriate physiological auto- and paracrine circuitries of growth factors and cytokines for

their own promotion (see above and in ref. 49). So it is with the syncytin system. Syncytin-1 is over-expressed in human endometrial carcinoma cells and induces cancer cell to cancer cell fusion. Steroid hormones elicit the production of TGF-ß1, 3, which are inhibitory to syncytin-induced cell fusions (295). Human breast cancer cells fuse with autologous vascular endothelial cells under the effect of syncytins (296). Such fused cells may participate in the formation of new blood vessel walls (neoangiogenesis; vascular mimicry, see above). Here, the receptor ASCT (neutral transporter of amino acids alanine, serine, cysteine) (297) expressed by both cancer cells and vascular endothelial cells to interact with syncytin molecules in order to mediate the cell fusions (296). Prostate adenocarcinoma cells over-express the ASCT receptor and show increased malignancy (but not known if due to stimulation by syncytin) (298).

Therapeutic cell fusions. Premature chromosome condensations in patients with leukemias and cancers represent naturally fused cells in these disease entities (299). When bone marrow-derived cells regenerate hepatocytes, it is with fusion between the targeted progenitor cells that leads to the regeneration of the hepatic parenchyme (300-302). Bone marrow-derived myelomonocytic cells fuse with normal and transformed intestinal stem cell. Fused transformed cells gain malignant potentials. Fused normal cells regenerate enterocytes, goblet cells, Paneth cells and enteroendocrine cells (303). Myoblasts fusing naturally with myogenic cells undergo normal differentiation. When myoblasts were forced to fuse with non-myogenic cells by using fusogenic Rous sarcoma or Sendai viruses, myotube and myofibril formations were suppressed and normal myoblast differentiation was inhibited (304). In order to regenerate, damaged cardiomyocytes fuse with surrounding non-cardiomyocytes, or with hematopoietic progenitor cells (305). Bone marrow-derived cells spontaneously fuse with neural progenitor cells to induce the regeneration of Purkinje cells of the cerebellum (306).

Transplantation of cell nuclei between adult somatic cells and stem cells results in reprogrammed recipient cells differentiating in the desired direction. When the transcription factors responsible for the reprogramming event are identified and cloned, the responsible genes can be retrovirally transduced (transfected) into adult somatic cells, thus generating pluripotent stem cells (307,308). These events may occur in nature during the processes of random or directed cell fusions. Blastocyte-derived embryonic stem cells possess the potency to regenerate the cell populations of the three layers: ectoderm, mesoderm and endoderm, whereas stromal stem cells already residing in the bone marow limit their differentiation into only connective tissues (the mesenchymal cells: adipo- myo-, cardiomyo-, osteo, and chondrocytes) and some neurocytes (the Purkinje cells, see above), raising the question if sarcomagenesis originate from these stem cells (see above). Bone marrow-derived myelomonocytes fused with damaged parenchymal cells may regenerate the parenchymal organ (liver), but when fused with transformed tumor cells, enhance the cells' malignant potentials (see above).

Long surviving allogeneic bone marrow transplant recipients developed squamous cell carcinomas originating in the oral mucosa which was chronically inflamed by graft-versus-host disease (GvHD). However, the tumor cells were those of the bone marrow donors (309). The erudite discussion does not consider donor-recipient cell fusion in the origin of these cancers (what this author proposes as a probability). In pregnant women with melanoma, fetal cells enter the lesions and form lymphatic vessels, thus probably contributing to increased incidence of lymph node metastases (310). No consideration was given to fetal-maternal cell fusions (see above).

Dendritic cell tumor cell chimeras as cancer vaccines. World-wide efforts take use of fused human DC-tumor cell vaccines. In order to avoid a tolerogenic presentation of tumor antigens (erbB2) by immature DCs, innate immune faculties, the signaling pathways of TLRs, had to be enlisted. Of the TLR signaling pathways, myeloid differentiation factor 88; IL-1 receptor-associated kinase-1 (IRAK-1), and TLR4 cytoplasmic domain, IRAK-1 could induce IL-12- and TNFα-mediated immune reactions. $CD4^+$ immune T cell anti-erbB2 responses were induced by the chimeric (ligated) anti-erbB2 (single-chain immunoglobulin)/IRAK-1 receptor (311).

Viral oncolysates prepared from a Ewing's sarcoma cell line were used for active tumor-specific immunotherapy in the 1970s (210,312,313). Since then, human cancer immunotherapy has advanced phenomenally. It is not the apoptotic, but rather the necrotic tumor cells, whose antigens are expressed by DCs in an immunogenic manner (49,314). It is not the intact host, but rather the pre-irradiated host that responds better to immunotherapy, be it vaccination, or adoptive immune lymphocyte infusions. In the host pre-irradiated with low doses (200-400 rads), the intestinal walls become penetrable for the bacterial flora and in response in the mesenteric lymph nodes the entire innate immune system goes into high alert (49,314,315). Against expectations, pre-irradiated hosts rejected intraperitoneally injected lymphoma cells better than did intact recipients (316). The National Cancer Institute USA includes low dose irradiation in its adoptive immune lymphocyte therapy protocols and reports improved results (317).

The Ewing's sarcoma EWS/ETS oncoprotein enhances the DKK 2 promoter (v-*ets*, E26 avian erythroblastosis retroviral oncogene's cellular counterpart c-*ets*; DKK, dickkopf gene first discovered in the drosophila; regulator of WNT-catenin signaling, see above) (318). Ewing's sarcoma was the target of DC-based immunotherapy in China. DCs were electrofused with Ewing's sarcoma cells, or pulsed with tumor cell lysates containing the EWS/FLI1 (Friend leukemia virus integration 1) oncogene. Human peripheral blood mononuclear cells were maintained in the peritoneal cavities of systemic combined immunodeficiency-afflicted SCID mice. Immune T cell clones reactive with Ewing's sarcoma cells emerged in this cell population in mice inoculated with the chimeric DC-tumor cell vaccine. SCID mice with Ewing's sarcoma-reactive human immune T cells rejected Ewing's sarcoma xenografts (319-321).

In chronic lymphocytic leukemia (CLL), DCs phagocytose apoptotic leukemia cells. Enriched populations of these DCs are used in Stockholm as Apo-DC vaccines administered with or without cyclophosphamide to patients with CLL (322). In patients with chronic myelogenous leukemia

(CML) relapsing after stem cell transplant, donor lymphocyte infusions may induce remissions, through alloreactive graft-vs-leukemia reaction with or without GvHD. Complete molecular remissions without GvHD were achieved in recipients with complete donor-chimeric T cell and DC subsets (323).

A melanoma vaccine consisting of autologous DCs matured by GM-CSF and IL-4 and pulsed with autologous melanoma cell lysates and potentiated with the adjuvant keyhole limpet hemocyanin was administered with or without low-dose IL-2 to patients with metastatic melanoma. Immune reactions were induced, but no clinical responses were observed in Ann Arbor, MI, USA (324). However, this vaccine was not a chimeric vaccine. In contrast, the allogeneic melanoma cell lysate TRIMEL loaded on autologous DCs effectively immunized patients with stages III/IV melanoma in Santiago, Chile. Anti-melanoma cell immune reactions of the vaccinated patients correlated with clinical responses. The immune reactions consisted of delayed hypersensitivity skin reactions to autologous tumor lysates and in the reduction of circulating $CD4^+FoxP3^+$ regulatory Treg cells. The clinical responses consisted of stabilizations of disease (progression-free long survivals): 33 months in immunoreactive patients versus 11 months in non-reactive patients (325). The German melanoma-allogeneic DC hybrid cell vaccine induced 1 complete and 1 partial remission and six stabilizations of disease in 17 stages III/IV vaccinated patients (326). Of 626 patients with advanced melanoma receiving DC-based vaccines, peptide vaccines induced the best clinical responses; but without clear correlation with immune parameters measured *in vitro*. Unexpectedly immature DCs with adjuvant-potentiated autologous antigens induced the better clinical responses (327). Immature DCs are considered inducers of tolerance. However, when myeloma cells were fused with immature and mature DCs, the immature DCs matured and expressed IL-12 and IFNγ, inducers of Th type-1 immune reactions (328). Metastatic melanoma is still considered to be an incurable disease; treatment protocols launching a concerted synchronous attack on the tumor cells, their blood supply, and their supportive microenvironment are 'eagerly awaited' (329).

As to adenocarcinomas, allogeneic breast cancer cell lines are being fused with autologous DCs for vaccination. MHC-restriction, to induce specifically immune T cells with cytokine release and induction of immune reactions to HER2/*neu* antigen (human epidermal growth factor receptor-2) hold these vaccines 'to be good candidates' for clinical trials (330,331). For ovarian carcinoma therapy, autologous tumor cell and autologous DCs are fused with PEG. In an MHC class II-restricted fashion, the vaccines induced immune T cell responses (332). Human DCs cultured with GM-CSF and IL-4 matured, but fused only in a low success rate (17%) with the human hepatocellular carcinoma cell line HLE; these 'dendritomas' are candidate vaccines (333).

Healthy volunteers donated peripheral blood monocytes for the generation of DCs in cultures with GM-CSF, IL-4 and TNFα. Electrical pulses were used to fuse the allogeneic DCs with the patients' kidney cancer cells. The fusion efficiency was 20%. Ten of 21 vaccinated patients responded with an immune reaction consisting of rising CD4/CD8 IFNγ-producer T cells. Two patients experienced partial response and in 8 patients the course of the disease stabilized (334). Patients with kidney cancer yielded tumor cells and peripheral blood-derived DCs for fusion. MHC-restricted tumor-specific immune T cell reactions were induced in the vaccinated patients. The kidney cancer antigen MUC-1 was expressed by the hydridomas. One of 10 vaccinated patients responded with partial regression of lung metastases and in 6 patients disease progression halted and stabilized the disease lasting for over 1 year (335).

The most effective technology of chimeric DC-tumor cell 'therapeutic' vaccinations against metastatic cancers has not as yet been discovered (336). Maybe, IFN-deprived tumor cells accept oncolytic viruses and repond to vaccination with viral oncolysates (see above). May be, virally fused DC-tumor cell chimeras will express tumor antigens in a more immunogenic manner and release oncolytic viruses within the tumor (242,243).

CD8 T cell-induced ETM transition and malignant transformation of human breast cancer stem cells. The exquisite susceptibility of human breast cancer stem cells to reoviral oncolysis. This author in the monograph 'Cytolytic Immune Lymphocytes...' elaborated on regulatory T (Treg) cells, suppressors of immune T cells and NK cells, and their attraction to tumor masses by the chemokine and its receptor, stromal-derived factor-1 (SDF-1; CXCL12, CXCR4) (49). Breast cancer neoantigen expressions (HER2/*neu*) (abbreviations: human epidermal growth factor receptor, HER; rat *neu* oncogene in ethylnitrosourea-induced neuroglioblastoma, homologous with erbB avian erythroblastosis retroviral oncogene v-*erb*; c-*erb* encoding epidermal growth factor receptor; human locus for HER2/*neu* mapped to 17q21.1) induce the generation of tumor-reactive immune T cells (337). However, continuous antigenic stimulation (as a physiologic defense against immunological overreaction converting into a pathophysiological counter-reaction) induces the generation of Treg cells (338). The paradoxical situation may arise, in which breast cancer tumors infiltrated with T cells in a reversed $CD4^+/CD8^+$ ratio, release cancer cells with increased metastatic potency to the regional lymph nodes (339).

Recently recognized is a new subclass of TGFß-producer $CD8^+$ T cells emerging to induce mouse and human epithelial breast stem cells to undergo ETM transition, and to gain malignant potentials in the process (340). In the view of this author, these $CD8^+$ T cells appear to have committed high treason against their host and hereby the proposal is made that they should be referred to as traitor/transforming T cells (T/T T cells). The so-called T/T T cells are under the control of newly discovered gene product proteins, which promote ETM transition (see above). These are the interleukin-like ETM transition inducers (ILEI), which require co-operation with the Ras oncoproteins to induce ETM transition of breast tubular cells, or hepatocytes, resulting in the emergence, during this process, of breast cancer and/or hepatocellular carcinoma cells (341,342). The subverted *snail* gene product proteins (see above), and TGFß and TNFα are the major promoters of the ETM transition (340-342).

The ancient protein family with two well conserved glycine residues (GG proteins) originated in the phage gp35 long tail

fiber hinge connector (343). The mammalian family of these proteins includes the FAMs (family members), especially the FAM3C gene product proteins (344,345). The 'osteoblast protein' FAM3C is strongly expressed also in pancreatic adenocarcinoma cells (345). In adition to, or independently from the *neu* oncogene (337), mouse breast cancer cells transformed by the Ras oncoprotein (rat sarcoma oncogene products, Kirsten or Harvey) undergo ETM transition under the effects of TGFß and ILEI, and express the anti-apoptotic protein Bcl-2 (346,347). The FAM3C gene (family member C sequence similarity 3) mapped to 7q31 is highly conserved in mammals; its gene product protein regulates cell differentiation in embryogenesis (344,348). The four FAM proteins (3A-D) belong to a new four-helix-bundle cytokine family and are widely expressed in vascular endothelium (FAM3A and B), in the placenta (FAM3D), and in the endocrine islet cells of the pancreas (FAM3B), and in breast parenchyma (FAM3C) (344). The human gene of FAM3B (PANDER = pancreatic-derived factor) is mapped to 21q22.3. This cytokine is induced in pancreatic ß-islet cells by IL-1ß, TNFα and IFNγ and consequentially it kills the insulin-producing cells by inducing their apoptotic death (349,350). This author expects to find such hidden FAM cytokines that would kill cancer cells upon similar immune stimulation. In contrast, in the breast, the gene product protein of the FAM3C gene is the ETM transition-inducer ILEI protein (340). Human breast cancer cells emerging from the process of ETM transition form mammospheres, a feature characteristic of epithelial stem cells (351). When a certain subclass of $CD8^+$ T cells (the T/T T cells) infiltrates breast cancer tumors, the formation of lymph node metastases is promoted (338,339). While HER2/*neu*-expressor breast cancer cells are immunogenic in their host of origin, HER2/*neu*-negative breast cancer cells arising after a long latency prevail, even in these immunoreactive hosts (346).

The tumorigenic human breast cancer cells could be identified as $CD44^+CD24^{-low}$ stem cells (352). According to the stochaistic theory of tumorigenesis, each malignant cell of a tumor cell population could initiate a new tumor; whereas, the hierarchy theory recognizes the heterogeneity of tumor cell populations and thus appoints only the tumor stem cells as generators of new tumor cell populations (353). It has now been recognized that the process of ETM transition de-differentiates the epithelial breast stem cell rendering it vulnerable to a Ras/MAPK mutation (mitogen-activated protein kinase). Thus the process of ETM transition generates breast cancer stem cells (340,347,351,354). In normal epithelial cells, genes of the mesenchymal phenotype (see above) are silenced. Are these genes liberated from epigenetic silencing and thus reactivated to induce ETM transition, or is there a gene excess transferred to epithelial cells through fusion of the epithelial stem cell with a mesenchymal (fibroblast, leukocyte, macrophage) cell?

The $CD44^+CD24^-$ human breast cancer stem cells exhibit high susceptibilty to oncolysis by reovirus (355). Ras-transformed tumor cells are deficient IFN-producers (242,356), thus are disposed to accommodating viral replication within them, whereas normal non-transformed host cells defend themselves by a vigorous IFN-response. Reoviral oncolysis is also effective in the case of certain malignant lymphoma cells (diffuse large B cell lymphoma), whereas other types of lymphoma cells are resistant (multiple myeloma, Burkitt's lymphoma, follicular lymphoma) (357). Stem cell preparations for transplants potentially contaminated with reovirus-susceptible tumor cells could possibly be purged of contaminating tumor cells (358). In the mouse, a recombinant replicating VSV (rrVSV) targets HER2/neu^+ breast cancer peritoneal implants. Viral oncolysis is significantly potentiated by the co-administration of antibodies to TGFß, IL-10, or CTLA4 (cytotoxic T lymphocyte antigen). The anti-tumor effects were mediated by CD4/CD8 immune T cells and foamy macrophages (probably practicing antibody-directed cytotoxicity and/or scavenging tumor cells debris) (359). Oncolytic viral therapy of human cancers may be rendered more effective when it is directed against cancer cells undergoing ETM transition, and administered in combination with anti-tolerance (anti-IL10; anti-CTLA4) and FcR-inducer monoclonal antibodies.

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