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REVIEW

Genes of ancient microtubule-stabilizing proteins traveled through pre-Cambrian Echinoidea to advanced life forms of dry land and ended up in the human genome as the fusion oncogenes-oncoproteins *eml1*/EML1-*abl*/ABL, and *eml4*/EML4-*alk*/ALK

JG Sinkovics

Department of Molecular Medicine, The University of South Florida Morsani College of Medicine, The Cancer Institute, St. Joseph's Hospital, Tampa FL, USA

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Abstract

The genes *eml1/4* of the Echinodermata microtubule-stabilizing gene product-like 1/4 proteins EML1/4 of the sea cucumbers (Holothuroidea) traveled through the evolutionary scale up to the human genome. Human cells malignantly transformed by oncogenes *abl* or *alk* enlist the protein products EML1/4 for the activation and protection of the gene product oncoproteins ABL or ALK against destruction by ubiquitination, and for gaining virulence and chemotherapy resistance. Neither the *abl* nor the *alk* genes act as oncogenes without fusion with another particular gene, such as *eml1/4* in this case. A large number of ancient but conserved gene product proteins chaperon, protect and enhance oncoproteins. These mechanisms indicate that ancient cell survival pathways exist conserved in the genomes of advanced multicellular diplo- and triploblastic hosts (including *Homo*). These genomic pathways are on special occasions constitutively reactivated in extant cells undergoing transformations for survival under adverse circumstances. Extant cells under threat react by re-living scenarios that characterized life forms in the primordial physico-chemical universe. In the clinical practice these cells are recognized as chemoradiotherapy-resistant cancer cells undergoing a process of retrograde immortalization.

Key Words: sea cucumbers; microtubule-associated proteins; oncogenes-oncoproteins; EML1/4 proteins

Introduction

Paleologists presume that in the precambrian environment ribozyme-like nucleotides coexisted with precellular ribozyme-armed ribosomes. Complex hypercycling RNA nucleotides deriving from hot subterranean vents travelled freely between these units. The RNA molecular proto-genes were sought after, were competed for, and were readily accepted in the working machinery of the targeted ribosomes. The precellular ribosomes contained tRNA precursors that could align amino acids in disorderly chains without any major conflict between the gene product peptide conglomerates pre-existing in the host, and those newly synthesized by the guests. The phenomena of horizontal first RNA, then DNA gene transfers may be viewed retrospectively as the natural form of existence in the world of precellular ribozyme-armed ribosomes sharing and exchanging first RNA, and then DNA genomes. With the unresolved episodes of DNA's appearance, spheroplasts appeared and fused into multicellular colonies mediated by fusogenic viruses, in the category first of RNA, then of DNA phages. Such a candidate virus is an extant fusogenic DNA phage of the protoplast mollicute *Acholeplasma laidlawii* (Sinkovics, 2016). Acholeplasma cells still release their primordial exosomes and lytic, temperate or fusogenic viruses (phages). All cells do, but especially cells undergoing malignant transformation in their multicellular host, exude an abundance of exosomes, as a signature for their atavistic return to the primordial life style of their ancestors. The first eukaryotes might have been formed by virally fused prokaryota and crenarchaea spheroplasts. Endosymbiosis with proteobacteria of the pre-fused spheroplasts formed the mitochondria in ancestral eukaryota in all categories of Animalia. Endosymbiosis of the pre-fused spheroplasts with cyanobacteria formed the chloroplasts in ancestral eukaryota of all categories of Plantae. Remnant members of a precellular virus world entered the primordial spheroplasts and their derivative fungal, plant and animal cells, thus creating the obligatorily

Corresponding author:
Joseph G Sinkovics
Cancer Institute St Joseph's Hospital
3001 W Dr Martin Luther King Jr Blvd
Tampa FL USA 33607
E-mail: Sinkovi.Joseph@baycare.org

virus-carrier three domains of life in the present era of Archaea, Bacteria (Prokaryota) and Eukarya (Eukaryota). The ancestors of the extant megavirales sealed their intimate relationship with ancestral amoebae. The liberal coalescence with alien genes has become strictly constricted, and the integrity of the individual DNA genomes became heavily guarded. The formerly practiced liberal horizontal gene transfers exerted major influence on, but without actual up-rooting, of the natural vertical course of evolution (extensively referenced in Sinkovics 2011, 2015, 2016).

In the present article, the evolution of certain microtubules from archaea and prokaryota will be followed through echinodermata eukaryotes, where they acquired their stabilizing proteins, EML1/4 (echinoderm microtubule-associated protein). It will be shown that a vital physiological system installed over three billion years ago remains conserved throughout evolution, as it becomes part of a retrograde immortalization process in extant multicellular eukaryotic hosts, including Homo. This process appears in the form of fusion oncogenes/oncoproteins in the human genome diagnosed at the clinics as the EML1-ABL malignant lymphoma, and the EML4-ALK lung non-small-cell adenocarcinoma.

In a most remarkable initial study (Bermudes *et al*., 1994), prokaryotic microtubules were observed by electron microscopy, but their tubulin protein composition was not immediately identified. These structures are already operational in the lower ranks of prokaryota: the Gram-positive mycoplasma *A. laidlawii* contains them. Cyanobacteria, the ancestors of chloroplasts in algae and plant cells, possess microtubules. If the term gamma subdivision of purple bacteria included proteobacteria, the ancestors of mitochondria in animal cells, the protofilaments and tubules reacting with antitubulin antibody were present in them. In spirocheta, heat-shock proteins cross-reacted with anti-tubulin antibodies, but Hsp-independent microtubules also appeared (Szathmáry, 1987). The contribution of microtubules, including flagellary undulipodia, from spirochetes to ancient eukaryota might have occurred from phagocytized spirochetes, or through routes of ectosymbiosis with spirochetes. Spirochetes, especially *Spirochaeta bajacaliforniensis* harbor longitudinally aligned tubulin microtubules of antitubulin antibody immunoreactivity (Bermudes *et al*., 1987). If the first eukaryota emerged from virally induced fusion of a mycoplasma cell (a fusogen phag-carrier *A. laidlawii*) with crenarchaeal spheroplasts (Sinkovics, 2016), the eukaryotic microtubules are of bacterial derivation. The similarity of protofilament interactions of bacterial and eukaryotic cells reveals that the bacterial microtubules represent the primordial structures that preceded eukaryotic microtubules evolutionarily (Pilhofer *et al*., 2011).

The ancient microtubular filamentous temperature-sensitive Z ring proteins (FtsZ) initiate and carry out the separation (by membrane abscission) of the daughter cells in the processes of cell divisions. Energy derives from the hydrolysis of GTP to GDP. Recruited proteins synthesize the new cell wall of the daughter cell. Single-stranded protofilamental subunits form the arrangements for the circular Z rings consisting of multistranded structures of microtubules. The Z rings are capable of exerting scissor-like contractile force. The Z rings form a scaffold for accessory proteins recruited for the separation of the daughter cell. The tubulin proteins of archaea and prokaryota become actin-myosin proteins in eukaryota. The FtsZ system is shared by prokaryota and crenarchaea represented by *Sulfolobus acidocaldarius* (with the exemption of Thermoproteales). The three-genes cdv cell division operons and CDV proteins, and the endosomal sorting complexes are required for transport (ESCRT) of molecular cargo, thus contributing to membrane remodeling, cellular abscission (separating the membranes by cleavage of two connected cells), and to multivesicular body biogenesis. These two systems are shared in crenarchaea and eukaryota (Lindås *et al*., 2008). Some FtsZ homologs (RepX from the plasmid pXO1 of *Bacillus anthracis*; TubZ from the virulence plasmid pBtoxis of *Bacillus thuringiensis*) are the replicators of the plasmids (Makarova and Koonin, 2010). The representative of a new crenarchaea-specific FtsZ-like 1 subfamily is the unique ESCRT-III-interacting tubulin protein of the crenarchaeon *Sulfolobus solfataricus* (after the volcano Solfatara di Pozzuoli at Naples, Italy). *Pseudomonas fluorescens* yielded another new FtsZ-like 2 domain that conserved all motifs of the nucleotide-binding loops, but without a coiled coil domain (which is present in the FtsZ-like 1 element) (Makarova and Koonin, 2010). The orderly assemblage of the FtsZ formation in bacteria could be physiologically inhibited by the Min system (discovered in *E. coli* minicells) and by nucleotid occlusion (NO) (Rowlett and Margolin, 2015). To the call to Google and Wikipedia for a list of FtsZ inbibitors in pathogenic bacteria and in eukaryota undergoing malignant transformation, a long lists of natural and synthetic molecular inhibitors appear including berberine, colchicine, chrysophaentin, curcumin, genistein, quercetin, plumbagin, resveratrol, sanguinarine, taxanes, totarol, and vincristine/vinblastine, etc.

The extremely acidophilic (optimally growing at pH 2 on temperature 60 °C), autotroph, methanotroph (using methane as its sole source of energy), *Methylacidiphilum infernorum* replaced its lost genes by horizontal receipt of numerous new genes, first from archaea, then from eukaryota, including FtsZs, but without synthesizing any microtubules; it fixes formaldehyde; and exempted itself of prophages due to an active CRISPR-associated system (clustered regularly interspersed short palindromic repeats). Its publication received extraordinary reviews (Hou *et al.,* 2008). In one particular case, a Verrucomicrobia other than *M. infernorum*, the *Prosthecobacter dejongeii*, has become the carrier of the *btub*A/B microtubule-encoding genes, whose gene product microtubular proteins are not of prokaryotic, but of eukaryotic structural and functional entities. Thus, in this case (or may be in all cases), the primitive αβ-tubulin genes entered after their duplication in a *P. ancestor* from a primordial eukaryotic cell possessing proto-tubulins assembled into microfilaments (Schlieper *et al*., 2008; Martin-

Galiano *et al.,* 2011). Thereafter the tubulin heteropolymers have undergone two separate but related evolutionary lines, one prokaryotic, one eukaryotic, both preserving their GTPase-activating domains.

Results

The primordial Echinodermata originated as crinoids in the Precambrian sea 600 mya. Extant Echinoidea and Echinozoa are represented by the sea cucumbers (Stichopus; Dendrochirotida; Holothuroidea), sea urchins (*Strongylocentrotus*), seastars (Asterozoa) and sandfish, sand dollar (Holothuriidae; Clypeasteroida; *Dendraster*). Echinoidea larvae and embryos form from the unison of haploid sperm-fertilized haploid eggs (the sea urchin meiosis of Oscar Hertwig, 1875). The dividing Sexualzellen, Keimzellen, Urkeimzellen, Stammzellen carry the Keimplasm of August Weismann, 1883 - 5 (while Gregor Mendel's articles rested on the shelfs of Charles Darwin, unopened and unread). The Keimplasm is understood to consists of chromosomes packed with DNA genes interspersed by RNA introns. The first three divisions of the Echinoidea zygote provide eight large cells of equal size. The fourth division yields macromeres and micromeres: LMics, large micromeres encoding the larval skeleton, that is the tubulovesical structures included; sMics, small micromeres undergoing the fifth cell division yielding mesomeres for the ectoderm and endoderm of the entire embryogenesis, and micromeres (sMics) for the germ line of the adult organism. The Wnt8/Notch/APC (wingless; integrated; adenomatous polyposis coli)/Dishevelled/CSK (casein kinase) and GSK3β (glycogen synthase kinase) pathway with intranuclear β-catenin activating the tcf/TCF (T cell factor), characterize the micromeres cells. GSK-phosphorylated cytoplasmic β-catenin is degraded by ubiquitylation; unphosphorylated β-catenin is transferred in micromere nuclei. At the 5^{th} cleavage, histone 3 lysine 9 is trimethylated (H3K9me3), thus silenced. Both LMics and sMics are needed for the formation of the mesenchymal blastocoel. LMics direct the metamorphosis of the archenteron gastrula of the larva into the adult rudiment, from which the adult organism develops. Calcium-transporting proteins maintain currents for communications between the cell organelles (endoplasmic reticulum, lysosomes, mitochondria). This article contains numerous illustrations in color, regretfully not reproduced here (Wessel *et al.,* 2014). Note: in vertebrate mammalian (including human) cells undergoing the so-called malignant transformation, due to deficient APC/GSK, unphosphorylated β-catenin is transferred into the nucleus, where it activates oncogenes, tcf/TCF in particular (referenced in Sinkovics, 2015, 2016).

The Hox (homeobox) gene cluster for bilateral to pentameral body patterning in the superphylum Deuterostomia (Cnidarian hydra, sea anemone, jellyfish) and Echinodermata, from ProtoHox to Hox and ParaHox anterior and posterior genes evolved and duplicated way before the divergence of these basal animals. In some cases, ParaHox is more involved in neurogenesis than in pre-bilaterian axis determination (Ferrier and Holland, 2001; Long *et al*., 2003; Chorrout *et al*., 2006; Quiquand *et al*., 2009; Ikuta, 2011; Byrne *et al*., 2016).

The longest living animals are among invertebrate taxa, especially those practicing reverse ontogenesis (Hydrozoa; Meduzoa, referenced in Sinkovics, 2016). Individuals of the gold and black corals Gerardia and Leiopathes, the clam Arctica of Island, the tube worm Lamellibrachia, the demosponge *Astrosclera* live hundreds even thousands of years (referenced in GIGA Community of Scientists, 2014). It remains unresolved what genomic constitution is responsible for the lack of senescence and the consequential life span extending over a century for the red sea urchin *Strongylocentrotus franciscanus,* in opposition to the four years life span for the green *S. variegatus*. Of mammalian species, the naked mole rat (*Heterocephalus glaber*) and the bat *Myotis brandtii* live long in absence of an aging process or neoplastic transformations (referenced in Sergiev *et al*., 2016 and Sinkovics, 2016).

The complement cascade and its interactions, or the lack thereof, with activator or inhibitor microRNAs evolved in lipopolysaccharide-responding coelomocytes of *Strongylocentrotus* (spu-miR) and in its relation, in the sea cucumber *Apostichopus japonicus* (AjC3); thus, the technology is available for further studies (referenced in Zhong *et al*., 2015). The *Strongylocentrotus*' major contribution is to the evolving recombination activating genes (RAG1/2) of adaptive immunity. The ancestors of these Transib transposon-mediated V(D)J transposition-catalyzing protein-coding genes reside in the genomes of the sea urchins *Strongylocentrotus* and Lytechinus (Fugmann, 2010; Kapitonov and Koonin, 2015). The target of the RAG transposases, the V(D)J sequence, is not present in the sea urchins; it appears first in the gnathostomata sharks. This particular genomic sequence might have been acquired by an ancestral herpesvirus Candidatus EBV (Epstein-Barr virus). The V(D)J-like sequence inserted into the EBV genome was discovered by Niller and Associates, in 2004. The paleo-immunology concerning the virally mediated insertion of the elements of the adaptive immune system into chondrichthyes ancestral cartilaginous sharks have been discussed by Dreyfus in 2009 and 2011; and by Sinkovics in 2011 and 2016. The sea urchins harbor numerous inserted active and inactivated retroviral elements acquired and propagated vertically and horizontally (Gonzales and Lessios, 1999).

Sea urchin and sea cucumber coelomocytes are primarily phagocytic amebocytes. The high diversity of their cDNA responses indicates that versatile immune responses were operational in the immunoglobulin-free era of native immunity. At the level of the sea squirt (*Ciona*) and the sea urchin (*Strongylocentrotus*) operational chemokine ligands and their receptors were non-detectable (de Faria and da Silva, 2008; Xue *et al*., 2015). The *Ciona* operates two toll-like receptors (TLR), but the *Strongylocentrotus* is well endowed with some 222 TLRs, which are paralogous with the TLRs of the

Amphioxus (*Branchiostoma floridae*) (Satake and Sekiguchi, 2012). In the sea cucumber (*A. japonicus*) two TLR genes were sequenced. AjTLR3 and AjToll were 3484 bp and 4211 bp, expressed leucine-rich repeats, and extended transmembrane into the cytoplasm. The two TLR genes were widely expressed, but in different extent in various tissues, including dominantly the coelomocytes. These receptors responded to LPS, peptidoglycans, polyinosinic and polycytidylic acids and zymosans implying broad responsibility to Gram-positive/negative bacteria and dsRNA viruses (Sun *et al*., 2013).

The Sp185/333 gene cluster encodes highly diversified gene product protein reactions in six element patterns to heat-killed marine bacteria; these reactions were restricted to distinguished cell lines. Thus individual phagocytes produced mRNAs for uniform protein (Buckley *et al*., 2008; Terwilliger *et al*., 2008; Majeske *et al*., 2014). The presentation of the major histocompatibility complex class II-restricted antigens in vertebrate mammalians is regulated by the enzyme gamma interferon-inducible lysosomal thiol reductase (GILT). The sea cucumber *Stichopus monotuberculatus* expresses a 1529 bp GILT protein of molecular weight 23.8 kDa. Its gene contains four exons and three introns. The protein displays NFκB- and IFNγ-binding sites. Endotoxin LPS upregulates the expression of this GILT, the initiator of innate immune reactions (Ren *et al*., 2015).

The Echinoderm microtubule-associated protein-like 1/4 activate and protect the human oncoproteins ABL in acute T cell leukemia, and ALK in non-small-cell lung adenocarcinoma

The echinoderm microtubule-associated protein (EMAP) family is represented by one single member from Echinodermata up to *Caenorhabditis* and *Drosophila* (EMAP-like protein ELP-1). By the vertebrate mammalians, the family, EMAPs expanded to five members (EML 1-5). In echinodermata, they are present in centrosomes and regulate the eccentric location of the germinal vesicle in oocytes during meiosis (Suprenant *et al*., 1993; Hamill *et al.,* 1994; Miyazaki *et al*., 2006; Houtman *et al*., 2007). The reader is encouraged to view in original the illustrations of the Hamill and Suprenant articles.

Microtubule-associated proteins (MAP) manufactured in the ribosomes, in general regulate microtubule formation and stabilization, thus the functioning of the entire cytoskeleton. The echinodermata EML4 gene analogs reappear in the *Caenorhabditis* as its elp-1 gene (Hueston *et al*., 2008), and in the drosophila as its doublecortin-domain containing echinoderm-microtubule-associated protein ortholog (Bechstedt *et al*., 2008). The genomic locus of the human EML4 gene (Gene ID 27439; also known as gene *ropp* 120 restrictedly-overexpressed proliferation-associated protein) is at the short arm of chromosome 2p21. The WD (tryptophan aspartic acid) repeat EML4 protein is of 981 aa with mass 108916 Da up to 120 kDa molecular weight (Pollmann *et al*., 2006). In the mouse genome, the ortholog of the human gene is at chromosome 17 (Gene ID 78798). The human EML4 is activated by phosphorylation of its serine/threonine residues. Its aa sequence 1-249 acts upon the microtubules in the mitotic spindle. HeLa cells with siRNA-deactivated EML4 proteins could not take up [3H]-thymidine and failed to develop mitotic figures. These HeLa cells showed an inactive microtubule network (Pollmann *et al.,* 2006). Of the newly evolved and recently discovered nuclear microtubule-binding proteins, EML3 is referred to as an echinoderm microtubule-associated gene product. It guards that microtubules to correctly align the chromosomes in the metaphase (as tested in HeLa cells). There are newly discovered spindle-accumulating EMAP family proteins: EML3 “poorly characterized” as such. The EMAP-like protein 70 (EML2) destabilizes and reorganizes microtubules in the M phase of the cell cycle (Tegha-Dungu *et al*., 2008). Some MAPs are described without claiming relationship to the echinodermata-related EMAP family (Orbán-Németh *et al.,* 2005). EMAP-like protein 5 (EML5) could be overexpressed in the human brain in both glial and neuronal cells. When overexpressed in the anterior temporal neocortex, it induces intractable epileptic seizures (Sun *et al*., 2015). Further discussions will be limited to the participation of *eml1* and *eml4* in the formations of human fusion oncogenes with proto-oncogenes *abl* and *alk,* thus encoding fusion oncoproteins EML1-ABL and EML4-ALK Abelson mouse leukemia retrovirus proto-oncogene; anaplastic leukemia kinase).

The cryptic translocation between genes *eml1*/EML1 at 14q32 and *abl*/ABL at 9q34 forming t(9;14)(q34;q32) occurred in a young female patient diagnosed with T cell acute lymphoblastic leukemia (T-ALL). This interaction results in the deletion of tumor suppressor cyclin-dependent kinase 2A (p16), and the expression of *tlx*/TLX1 gene-product protein, which is a human homolog of the drosophila tailless gene, a cell cycle driver, encoded in the human genome as nuclear receptor nr2e1/NR2E1 gene-product protein. Further upregulated pathways are ERK1/2 (extracellular signal-related kinase); STAT 5 (signal transducer and activatior of transcription); and the Lyn kinase (abbreviation of united Lck/Yes kinases: lymphocyte kinase of the Rous sarcoma retrovirus src family, and Yamaguchi sarcoma retrovirus kinase). The coiled-coil domain of the EML1 protein is incorporated in the naturally formed 190 kDa the fusion oncoprotein. Removal of the coiled-coil domain disabled the oncoprotein. The technology for this molecular diagnosis involved Fish (fluorescence in situ hybridization), RACE (5’-rapid amplification of cDNA ends polymerase chain reaction); constructs of open reading frame of exon 1 to 17 of EML1 amplified with primer EML1-F1 and -R. ABL was amplified with primers ABL1-F and –R. The EML1/del EML1 parts were ligated in the murine stem cell retroviral vector puromycin kit (Clontech, Palo Alto, CA). Imatinib sensitivity was tested for in cell cultures and expressed in growth curves. The *abl* proto-oncogene was known in other leukemias to fuse with genes *bcr* (breakpoint cluster region), *nup* (nucleopore-to-gene-promoter interaction), and *mll* (mixed lineage leukemia). In this case, the *abl* /ABL oncogene/oncoprotein inhibitor imatinib mesylate induced durable

complete remission. Figures depicting of the fusion oncogenes-*eml1/alb* are shown and referenced in the cited articles (De Keersmaecker and Huret, 2005; De Keersmaecker et al., 2005; Van Etten, 2005). The reader is encouraged to view these figures in original.

Amplifications of EGFR in adenocarcinomas, and FGFR1 in squamous cell carcinomas of the lung are common events treatable accordingly, with individually appropriate monoclonal antibodies, chemotherapeuticals, targeted therapy (gefitinib; erlotinib, imatinib; PD173074) and with small molecular inhibitors and monoclonal antibodies to neo-vasculogenesis. However, the recently emerged EML4-ALK-driven lung adenocarcinomas require specific considerations (Weiss , Sos, Seidel et al., 2011). The fusion oncogenes consisted of the inversion of the short arm of chromosome 2 juxtaposing the 5' end of the *eml4* gene with the end of the *alk* gene, so that intron 13 of the *eml4* gene fused with intron 19 of the *alk* gene. The encoded oncoprotein assumes three isoforms (versions or variants 1, 2 and 3). Variant 3 dominates in China (52 %); variant 1 inflicts Caucasians (75 %) (Zhao *et al*., 2015). All variants of the *eml4/alk* fusion genes replicate constitutively. Figures of the fused oncogenes *eml4/alk* are depicted in cited article (Choi *et al*., 2008). The reader is encouraged to view these figures in original (Karachaliaou and Rosell, 2014). All tumors with *alk*/ALK involvement are referred to as ALKomas (Mano, 2012),

These tumors only occasionally express v-Ki-ras2 (Kirsten rat sarcoma retroviral homolog), or EGFR additional mutations, because of the belief that these co-mutations are mutually exclusive. In cases of second co-mutations, crizotinib therapy fails to control the disease (Ulivi *et al*., 2015). In China, in addition to non-small-cell lung cancers of the well-recognized heterologous etiology of driver genes EGF-R, v-Ki-ras2, and v-raf (rat fibrosarcoma) oncogene homolog B (BRAF), tumors of EML4-ALK etiology not responding to erlotinib or gefitinib, but responding to crizotinib emerged. Most of these adenocarcinoma tumors were diagnosed in their advanced stages in young non-smoker women (Zhao *et al.,* 2015). Occasionally elderly smokers can be involved (Choi *et al*., 2008). In these patients, EGFR. KRAS and EML4-ALK rearrangements may co-exist (Yang *et al.,* 2016). Even when diagnosed in early stages, these tumors are considered highly malignant, due to their undifferentiated histopathology (Ren *et al.,* 2015). Otherwise, co-existence of the EGFR and KRAS mutations, if any at all, are expected to be very rare (Zhu *et al*., 2014). In India, the incidence of EML4-ALK lung cancers is 3 % (versus 33 % for EGFR mutation-induced lung cancers). These EML4-ALK tumors retained high sensitivity to crizotinib at 250 mg twice daily, as measured by 72 % progression-free survival at 7 months and 77 % and 64 % overall survival at 1 and 2 years (Doval *et al*., 2015).

Crizotinib is the specific inhibitor of alk/ALK and is the therapy of choice for the ELM4/ALK tumors. ERBB ligand (erythroblastic mouse leukemia retroviral growth factor human homolog) expressor EML4-ALK tumors gain crizotinib resistance (Kimura *et al*., 2015). EML4-ALK tumors express the CD133 stem cell marker and the extracellular signal regulated ERK pathway. These tumor cells undergo epithelial-to-mesenchymal EMT transitions (Guo *et al.,* 2015). When stemness-associated molecules rise: ALDH, aldehyde dehydrogenase, human sarcoma stem cell marker (Lohberger *et al.,* 2012); NANOG (never aging celtic tribe); OCT4 (octamer-binding transcription factor), the tumor cells acquire crizotinib resistance. In this case, rapamycin combined with crizotinib induces a synergistic action (Oh *et al*., 2015). A second generation *alk*/ALK tyrosine kinase inhibitor AP26113 (Ariad Pharmaceuticals) is in clinical trial at the University of Milano-Biocca, Italy (Ceccon *et al.,* 2015). Explanation: the human fusion oncogene/oncoprotein *npm-alk*/NPM-ALK (nucleolar phosphoprotein nucleophosmin) at t(2;5)(p23;q35) causes anaplastic large cell lymphoma. Retroviral transfer of the fusion oncogene into mice induces the lymphoma (Kuefer *et al*., 1997).

Discussion

The so-called cellular malignant transformation (the clinical diagnosis cancer) is considered here to be the manifestation of an inherent faculty of the original RNA/DNA complex consisting of a process referred to as a retrograde immortalization of individual stem and/or somatic cells in a highly organized multicellular host organism, including Homo. The process of cellular transformation clinically referred to as cancer is the expression of a backward genetic regression to the original level of evolution. That overheated over-irradiated and chemically imbalanced (mainly hyperacid due to an excess sulfuric acids) environment was populated by primordial multi-resistant cells. Those were the circumstances at the edge, the unicellular microorganisms, and the diplo- and triploblastic macroorganisms have had organized themselves into. Those cells were endowed with the faculties of reversed ontogenesis (as in Cnidaria/Medusozoa) and trans-speciation (sporulation of bacteria; encystation of Giardia; the dauer phenomenon of *Caenorhabditis*; existence in the state bordering autophagy in the life cycle of *Dictyostelia*; trans-speciation of the entire microtubular cytoskeleton in *Naegleria*) with full recovery (referenced in Sinkovics, 2016). A great deal of the ancient cell survival pathways remained conserved throughout evolution.

The heat shock proteins Hsp60s of Crenarchaeota appear as predecessors of eukaryotic Hsp, as their cell division pathway is eukaryota-like. The mammalian vertebrate descendants of the ancient heat shock proteins of the hyperthermic archaea *Archaeoglobus fulgidus* or *Haloferax volcanii* (Rohlin *et al.,* 1995; Cox *et al.,* 2008) now chaperon oncoproteins encoded in the human genome (Calderwood and Gong, 2012). The toxin expulsion pumps of the algae are used by malignantly transformed high level parenchymal cells of multicellular organisms (including *Homo*) to expel chemotherapeutic molecules (referenced in Sinkovics, 2015, 2016). Oncogenesis induced by extrinsic factors (oncogenic viruses) elicits strong immune defense reactions. Whereas, endogenously

induced by inserted retrotransposons, the so-called malignant transformation of individual selected cells receives full support consisting of vascularization; microRNAs-directed chemokines, lymphokines, cytokines; and growth factors of fibroblast- and M2 macrophage-origin (extensively referenced in Sinkovics 2015, 2016). Within certain circumstances, the host supports the retrograde immortalization of some of its selected cells.

Here, examples are provided, as the oncogenes/oncoproteins in a so-called malignantly transformed cell enlist the descendants of ancient microtubule stabilizing proteins (EML1/2) for rendering service to them consisting of their activation; protection against destruction by ubiquitination; gain of virulence and stem cell faculties; epithelial-to-mesenchymal transition (EMT); and gain of resistance against the chemotherapeuticals, including crizotinib.

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