INTRODUCTION

Malignant transformation is the process by which cells acquire the properties of cancer. The first successful malignant transformation in vitro was achieved with the polyoma virus on Syrian hamster embryo cells, followed by transformation with chemical carcinogens in the mid-1960th (reviewed in [1]). Reports of human cell transformation using viruses and viral oncogenes appeared only in the late 1970th [1]. In early 80th it was shown that immortalized NIH3T3 mouse fibroblast cell line, human embryonic kidney 293 cell line (HEK293), and human mammary epithelial cell lines (mainly HMECs and MCF10A). These cell lines have abnormal karyotypes and are prone to progress to malignantly transformed cells. This review is aimed at understanding the mechanisms of cell immortalization by different “immortalizing agents”, oncogene-induced cell transformation of immortalized cells and moderate response of the advanced tumors to anticancer therapy in the light of tumor “oncogene and chromosome addiction”, intra-/intertumor heterogeneity, and chromosome instability.

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blasts and human primary mesodermal cells introducing simultaneously three oncogenes E1A, MDM2, and H-RASV12. These cells formed colonies in soft agar and tumors in mice, but they and the majority of the tumors derived from them lacked telomerase activity, and telomere erosion was observed [10]. Authors have deduced that telomere maintenance is not obligatory for tumorigenic conversion. To the point, human primary melanomas show telomere maintenance as a late event in tumor progression (metastatic melanoma), thus, telomere maintenance/immortalization is associated with progression rather than initiation of melanoma [11].

Furthermore, like primary human cells, primary MEFs require combination of two “hits” to acquire the capacity to form tumors [9, 12–19]. There are also cases of a conversion of normal primary rodent [20–23] and human [24–27] cells to fully transformed cells with a single oncogene under specific experimental (significant overexpression of oncogene) and culture conditions. Culture conditions significantly affect proliferative (before senescence) [5] and transformation potential of cells [8]. For example, wild type MEFs grown in serum-free medium supplemented with defined growth components (EGF, PDGF, insulin, high density lipoprotein, fibronectin, and transferrin) were refractory to transformation by oncogenic RAS + E1A [7]. Moreover, RAS + E1A-induced chromosomal instability, colony formation and tumorigenesis of the p53 −/− serum free-MEFs also could be attenuated by treating the cells with the free-radical scavenger N-acetylcysteine [7].

Finally, humans live, on average, 30–50 times longer than mice and undergo about 10⁶ more cell divisions in a lifetime (10¹⁰ versus 10¹¹ mitoses) [4]. Nevertheless, epidemiological studies have revealed that the life-time risk of developing cancer is comparable in both species. About 30% of laboratory rodents have cancer by the end of their 2–3 year life-span and about 30% of people have cancer by the end of their 70–80 year life-span [4].

Thus, it seems that in vitro (and likely in vivo) transformation process may be fundamentally similar in rodent and human cells and be significantly affected by non-physiological culture conditions in vitro.

SENEGENCE

In contrast to germ cells and certain stem cells somatic cells have a limited lifespan, gradually slow in growth, and stop dividing, a process known as replicative senescence [28]. The finite replicative life span of normal cells in culture was first described approximately 50 years ago by Leonard Hayflick [29], and is often termed as the “Hayflick limit” [30]. The precise number of replicative doublings exhibited by cultured cells before they reach senescence depends on the species from which the cells are derived, the tissue of origin, and the age of the donor organism [31]. Cultured human primary fibroblastic cells generally display 50 to 80 population doublings (PD) [7, 32, 33], whereas explanted MEFs can divide just for 15–30 PD before undergoing senescence [5, 7]. Primary normal human astrocytes perform only about 20 PD before reaching senescence [34]. Human keratinocytes have an in vitro life span of 15–20 PD in serum-free chemically defined media, whereas keratinocytes grown on feeder fibroblasts proliferate for up to 50 PD [7, 32] and in F medium on feeder fibroblasts for up to 80 PD before senescencing [35]. Most published reports on cultured human epithelial cells have shown active growth for only 10 to 30 PD [32]. Significantly, simple changes in the culture conditions (defined growth factors instead of serum) could permit active growth of human mammary epithelial cells for up to 60 PD, whereas addition of oxytocin (endogenous antioxidant) gave about 20 PD of increased proliferation [32]. MEFs proliferate for more than 60 PD with no signs of replicative senescence under physiological oxygen levels (3% versus 21%) [7, 8]. Thus, primary cells undergo stress-associated senescence due to in vitro non-physiological standard culturing conditions, including disruption of cell–cell contacts, lack of heterotypic interactions between different cell types, the medium-to-cell ratio, persistent signaling pathways activation by mitogens, absence of appropriate survival factors, hyperoxia, and plating on plastic [5].

The process of senescence occurs both in vitro and in vivo. Cellular senescence in vivo is now recognized to play an active role as a tumor suppressor pathway [36, 37], in the loss of regenerative potential in aging tissues and in the pathogenesis of cardiovascular diseases [38]. Senescence in vitro is marked by the appearance of large, flattened vacuolated cells and characterized by the inability of cells to proliferate despite the presence of a steady supply of abundant nutrients, mitogens [39], ample room for expansion [33], and by the maintenance of cell viability/resistance to apoptosis and metabolic activity for months [37, 38, 40]. Once senescence is triggered, cells are not capable of re-entering the cell cycle or developing into tumors [36]. Moreover, senescent cells secrete a plethora of factors primarily involved in insulin-like growth factor and transforming growth factor β signaling, extracellular matrix remodeling, and inflammation. Altogether these secreted factors were referred to as the “Senescence-Messaging Secretome” or the “Senescence-Associated Secretory Phenotype” [33].

Senescent cells can be distinguished from pre-senescent, immortal, quiescent or terminally differentiated cells by histochemical detection of the biomarker senescence-associated β-galactosidase [41]. Senescence accompanies changes in nuclear morphology and formation of a distinct chromatin structure, called senescence-associated heterochromatic foci (SAHF). These foci are characterized by the accumulation of histone H3 trimethylated at lysine 9