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Research Article

SNEV overexpression extends the life span of human endothelial cells

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ABSTRACT

In a recent screening for genes downregulated in replicatively senescent human umbilical vein endothelial cells (HUVECs), we have isolated the novel protein SNEV. Since then SNEV has proven as a multifaceted protein playing a role in pre-mRNA splicing, DNA repair, and the ubiquitin/proteosome system. Here, we report that SNEV mRNA decreases in various cell types during replicative senescence, and that it is increased in various immortalized cell lines, as well as in breast tumors, where SNEV transcript levels also correlate with the survival of breast cancer patients.

Since these mRNA profiles suggested a role of SNEV in the regulation of cell proliferation, the effect of its overexpression was tested. Thereby, a significant extension of the cellular life span was observed, which was not caused by altered telomerase activity or telomere dynamics but rather by enhanced stress resistance. When SNEV overexpressing cells were treated with bleomycin or bleomycin combined with BSO, inducing DNA damage as well as reactive oxygen species, a significantly lower fraction of apoptotic cells was found in comparison to vector control cells. These data suggest that high levels of SNEV might extend the cellular life span by increasing the resistance to stress or by improving the DNA repair capacity of the cells.

Introduction

After a defined number of population doublings in vitro, normal human cells enter a state of irreversible growth arrest termed replicative senescence [1]. Although this growth limit on the one hand has been reported to play an important role as a tumor suppressive mechanism [2], the phenotypic changes associated with aged cells on the other hand are discussed to contribute to aging and age-related diseases, such as several types of cancer [3]. Therefore, the characterization of senescent cells is of utmost importance. Since cellular phenotype and physiology are determined by the repertoire of expressed and translated genes, several studies have been performed in order to isolate genes and

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proteins that are differentially expressed during aging of normal human cells [4–6]. Similarly, we have reported the identification of several differentially expressed genes, among which we found a protein that we termed SNEV for "Senescence Evasion Factor" [7], showing sequence similarity to the yeast Prp19/Pso4 protein, which has been characterized as essential yeast splicing factor [8]. Recently, we have been able to show that SNEV is indeed the human orthologue of Prp19 and as such also essential for the human pre-mRNA splicing reaction. SNEV forms at least homodimers, and, by interfering with this oligomerization, the splicing reaction is blocked in vitro [9]. Supporting its role as a human splicing factor, SNEV has been identified as nuclear matrix protein NMP200 [10] and as core member of the CDC5L associated protein complex by various proteomic approaches to characterize the spliceosome or related complexes [11–13].

In addition to its role as a splicing factor, SNEV has also been identified as a U-box protein showing ubiquitin E3 ligase activity in vitro [14]. Although its activity as E3 ligase has not been proven in vivo so far and the substrate is still unknown, a possible E3 activity in living cells is supported by our recent finding that SNEV directly interacts with the proteosome, without being an in vitro substrate [15].

Another highly interesting report on the function of SNEV was published recently, showing a possible role in DNA double strand break repair [16], a function that is supported by the notion that also yeast Prp19 is involved in DNA repair [17]. Especially this function inspired our choice to further investigate the role of SNEV during cellular aging, since uncapped, shortened telomeres associate with many DNA damage response proteins, and replicative senescence resembles the growth arrest induced by activation of DNA damage checkpoint response pathways [18]. Furthermore, besides telomere shortening and dysfunction, DNA damage per se, induced by exogenous or endogenous stress, may cause the entry into senescence [19]. However, the cellular response to stress situations can be variable, including induction of DNA damage response pathways leading to senescence or apoptosis as well as transient cell cycle arrest and DNA damage repair. Whichever the trigger for induction is, entry into irreversible growth arrest is executed by the p53/p21 and/or the pRb/p16 pathways [3].

Here, we report the follow up of our previous observation of differential regulation of SNEV mRNA in replicatively senescent endothelial cells. We show that less SNEV transcript is found in senescent cells of different tissue origins, while it is higher expressed in tumor cell lines when compared to normal cells and therefore seems to correlate with the replicative potential of cells. Therefore, we investigated the effect of increased amounts of SNEV on growth behavior of endothelial cells by generating stable SNEV overexpressing cell lines. These cell lines showed an extended life span in vitro that was not due to telomerase reactivation or altered telomere dynamics but rather to increased resistance against stress and lower levels of DNA damage. This potential DNA protective activity prompted us to analyze SNEV mRNA levels in tissue samples of breast cancer patients, and we found that high levels of SNEV positively correlated with the overall survival of the cancer patients.

Materials and methods

Cells and culture conditions

HUVECs were isolated from umbilical veins as described previously [20] and cultivated in gelatin (1% in PBS) precoated culture flasks in M199 medium (Biochrom) supplemented with 4 mM L-glutamine, 15% fetal calf serum (FCS, Hyclone), 200 μg/ml endothelial cell growth supplement and 170 U/ml heparin. HUVECs with extended life span (ESV) have been established using the SV40 early region [21]. Human diploid fibroblasts were isolated from skin biopsies [22] and were grown in DMEM/Ham's-F12 (1:1) supplemented with 4 mM L-glutamine and 10% FCS. Normal human diploid proximal tubular epithelial cells were isolated from renal tissue biopsies [22] and were grown under serum-free cell culture conditions. Immortalization of kidney epithelial cells (HK42/1) was achieved by introduction of the SV40 early region. Confluent adherent cultures were detached using 0.1% trypsin and 0.02% EDTA and were passaged with an appropriate split ratio of 1:2 or 1:4 once or twice a week depending on confluence and population doubling level (PD). Subsequently, cumulative PDs were calculated as a function of passage number and split ratio [6]. Normal lymphocytes were isolated from whole blood using Ficoll gradient and were activated by phytohemagglutinin.

All other cell lines were obtained from ATCC if not stated otherwise and were cultivated according to the suppliers' specification: HL-60 (human promyelotic leukemia), Molt 3 (human acute lymphoblastic leukemia, T-cell), AA-2 (WIL-2 human spleen EBV^+ B-lymphoblastoid cell line, National Institute of Allergy and Infectious diseases), MT-2 (human T-cell leukemia, National Institute of Allergy and Infectious diseases), A-498 (human kidney carcinoma), HEK293 (human transformed embryonic kidney). Additionally, 15 human breast cancer cell lines (Hs578T, AU565, SK-BR3, ZR-75-1, BT-474, Hcc1143, Hcc1937, MDA-MB-453, HTB131, Kpl-1, MCF-7, T47D, MDA-MB-468, Cal51, Cama-1, MDA-MD-231, MDA-MB-435) as well as 4 normal human breast cell lines (MCF-10A, MCF-10F, HMEC, Hs578Bst) were used in this study.

Growth arrest of quiescent normal cells was induced by contact inhibition. Therefore, the cells were grown under normal cell culture conditions and harvested 1 week after having reached confluence.

RNA extraction and Northern blotting

Total RNA from senescent, quiescent or exponentially growing cells was isolated using 1 ml TRIzol® reagent (Life Technologies)/5×10^6 cells, and mRNA was isolated using paramagnetic oligodT beads (Dynal) using standard procedures. SNEV cDNA was DIG labeled by PCR according to the manufacturer's protocol (Roche) and then used as probe on Northern blots. 10 ng probe/ml high SDS hybridization buffer was used, and hybridization was carried out overnight at 50°C. After stringent washing conditions, chemiluminescent signals were detected using CDP-star measured by the LumiImager (Roche). The Northern blots contained 12 μg total RNA/lane.