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Review

Redox control of viral carcinogenesis: The human papillomavirus paradigm ☆

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ABSTRACT

Background: Cervical cancer is the second most common neoplastic disease among women worldwide. The initiating event of such cancer is the infection with certain types of human papillomavirus (HPV), a very common condition in the general population. However, the majority of HPV infections is subclinical and transitory and is resolved spontaneously. Intriguingly, viral oncogene expression, although necessary, is not per se sufficient to promote cervical cancer and other factors are involved in the progression of infected cells to the full neoplastic phenotype. In this perspective it has been suggested that the redox balance and the oxidative stress (OS) may represent interesting and under-explored candidates as promoting factors in HPV-initiated carcinogenesis.
Scope of the review: The current review discusses the possible interplay between the viral mechanisms modulating cell homeostasis and redox sensitive mechanisms. Experimental data and indirect evidences are presented on the activity of viral dependent functions on i) the regulation of enzymes and compounds involved in OS; ii) the protection from oxidation of detoxifying/antiapoptotic enzymes and redox-sensitive transcription factors; iii) the suppression of apoptosis; and iv) the modulation of host microRNAs regulating genes associated with antioxidant defense.
Major conclusions: The resulting tangled scenario suggests that viral hosting cells adapt their metabolisms in order to support their growth and survival in the increasingly oxidant micro-environment associated with HPV tumor initiation and progression.
General significance: HPV can modulate the host cell redox homeostasis in order to favor infection and possibly tumor transformation. This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

1. Introduction

Cancer is a general term that comprises a number of clinically and biologically different conditions affecting almost any tissue in any organ and apparatus of humans. The observations made in humans as well as in either wild and experimental animals suggest that tumor development proceeds through a process recalling the Darwinian evolution: a succession of genetic and epigenetic alterations, each conferring one or more surviving advantage, leads to the progressive conversion of normal cells into neoplastic cells [1]. Although the path that cells takes along this complex process, called neoplastic progression, is extremely variable, nonetheless a few general traits emerge and an ultimate definition of cancer can be outlined. Cancer is a condition in which the fundamental mechanisms of growth control are largely disrupted while the growth ability is somewhat retained. A pathological cell is thus generated with a few essential alterations in cell physiology, namely (Box 1): independence from external proliferative signals; insensitivity to anti-proliferative signals; evasion from apoptosis; unlimited proliferative potentials; ability to sustain neo-angiogenesis; and tissues invasion and metastasis [2]. Each of these features (but not their underlying genetic/epigenetic mechanisms) may be also found in normal cells in specific life stages and none of them is either specific or sufficient for cancer definition. On the contrary, it is their simultaneous occurrence, in a patchwork of properties otherwise specific of distinct stages of cell life, to characterize cancer, which can be appropriately defined as a condition of combined pathological differentiation and de-differentiation.

2. Viruses are precious tools in modern cancer research

An astonishing complexity is undoubtedly the hallmark of cancer biology and the search for convenient simplified models has been a

☆ This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

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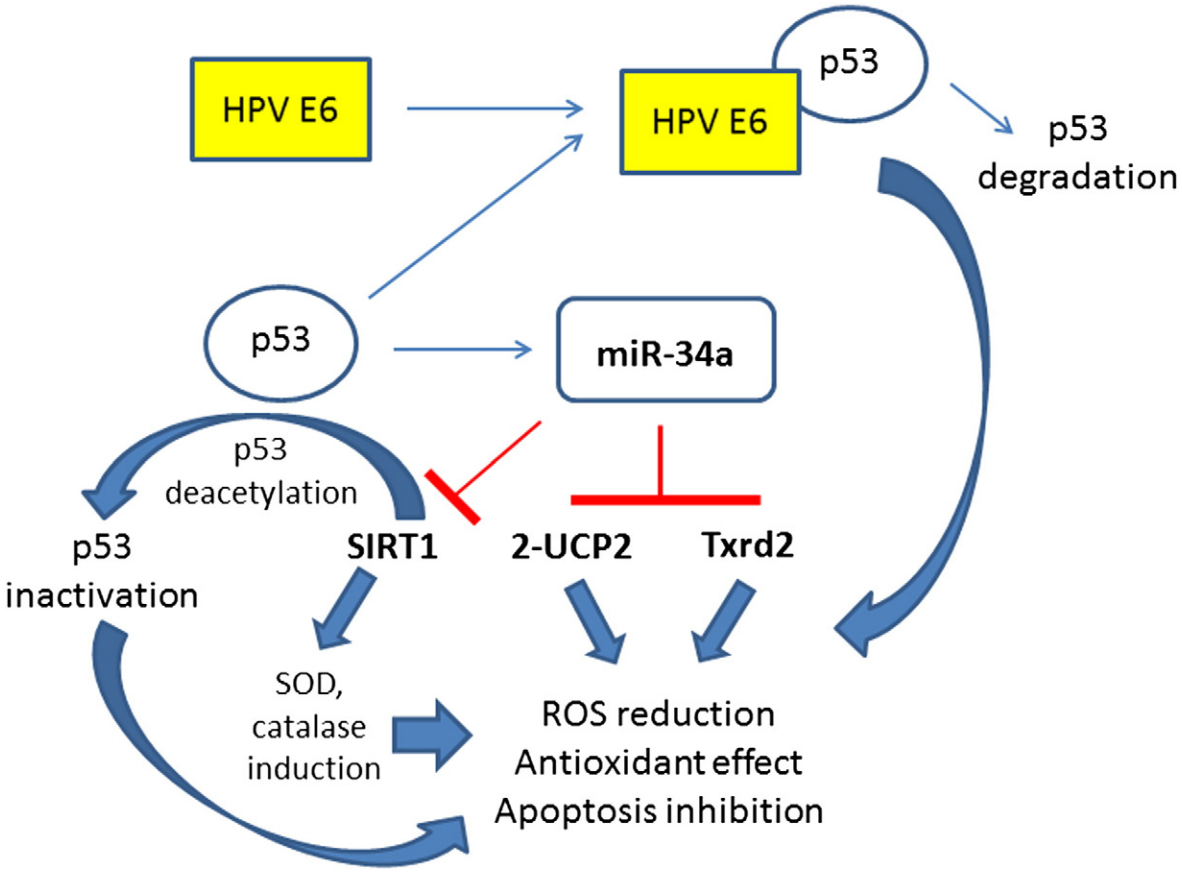


Fig. 1. Effects of miR 34a modulation by HPV E6. miR-34a transcription and expression are stimulated by p53. miR-34a induces apoptosis and ROS increase, through downregulation of Sirtuin 1 (SIRT1), uncoupling protein 2 (UCP2) and thioredoxin reductase 2 (Trxrd2). During HPV infection, viral oncogene E6 determines the degradation of p53. Consequently, miR34a is suppressed and SIRT1, UCP2 and Trxrd2 can exert their antioxidant and antiapoptotic functions.

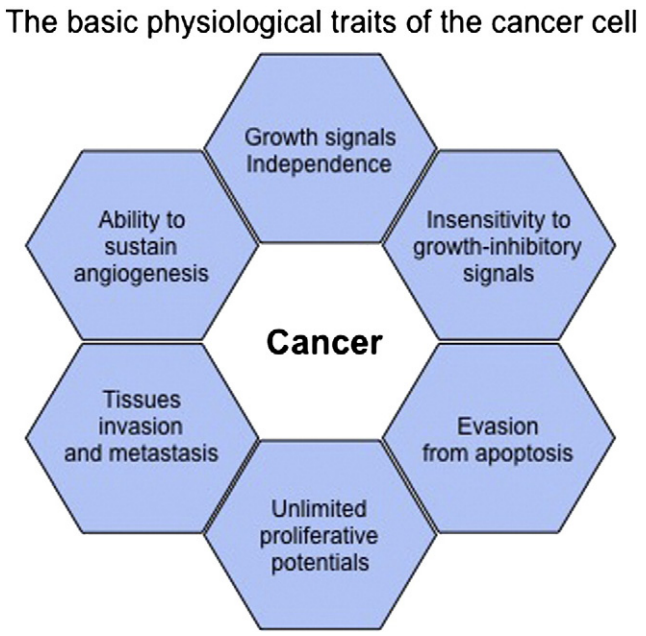
constant effort since the beginning of modern medicine. Accordingly the idea of viral carcinogenesis has always been a very attractive one. However, despite clear and sound evidences of viral induced neoplasia date back to the origin of virology [3,4] and despite viral oncology has since been central to modern cancer research providing profound insights into both infectious and non-infectious cancer [5], the majority of scientist remained skeptical about viruses as natural causes of cancer. The common perception changed drastically across the last three decades and currently viral carcinogenesis is an undisputed issue. Seven human viruses have been found to cause 10–15% of human cancers worldwide (Box 2) and studies are ongoing to take advantage from the readily identifiable targets for diagnosis, prevention and therapy these cancers offer. This process culminated with Nobel Prizes awarded in 2008 for the discovery by Harald zur Hausen of high-risk human papillomavirus (HR-HPV) strains that cause cervical cancers (Box 3) [6].

Among human oncogenic viruses, because of its specific biology HPV represents a particularly convenient tool in cancer research: i) HPVs induce a widely diffused cancer through the natural infection [7], ii) HPVs have a rather little genome whose structure and basic functions are fairly known [8], and iii) their oncogenic potentials are linked to the coordinated activity of three viral proteins whose specific activity is aimed to release the cellular control on proliferation, apoptosis suppression, and potentiation of the response to growth factors [9,10]. These perturbations create the conditions permissive for viral replication which, at the same time, happen to be conducive to cancer development; iv) the viral replicative cycle is in turn strictly controlled by the host, with the viral progeny maturation strictly synchronized on cell differentiation program [11]; v) the tumor initiation and progression, which disrupt the viral productive cycle, can be replicated in vitro allowing (at least from a theoretical point of view) the direct observation of the biochemical and molecular mechanisms driving cancer.

3. A short description of HPV biology

Although a detailed description of HPV structures and functions is out of the scope of the present work, a brief outline of the viral life cycle is of help to understand how the viral infection can shift into neoplastic growth. The virus particle, usually 50–55 nm in diameter, is composed of two proteins, L1 and L2, encapsidating a double stranded circularly closed DNA of roughly 8000 bp length coding for 7–9 genes [12]. All known genes are encoded in one DNA strand and transcription is unidirectional. The genome can be divided into three regions: the early (E) region, which is responsible for the pathogenicity of the virus, invariably includes the E1 and E2 genes and in most of cases includes the E6 and E7 genes too; the late (L) region, which invariably codes for late structural proteins L1 and L2; and a non-coding region (NCR) which contains the genetic elements for replication and transcription of viral genome [12–14].

Entirely based on sequence homologies many different types, species and genera of PV have been identified [15–17]. A remarkable feature of PVs is their strict species-specificity and, within a host species, their very strong predilection for specific cutaneous or mucosal district. Within such a classification, all human PV (HPV) are comprised in the genera: alpha, beta, gamma, mu and nu. Alpha PVs infect cutaneous and oropharyngeal/ano-genital mucosal districts; beta PVs are typically associated with latent/unapparent cutaneous infections in the general immunocompetent population; gamma PVs are associated with benign skin lesions and may be occasionally found in oral mucosae; mu PVs are usually isolated from benign proliferative lesions of the palmar/plantar



Box 1. The basic physiological traits of the cancer cell.

sites and nu PVs are found in other cutaneous lesions [18]. Within epithelial tissues the infection requires the availability of basal layer cells. Thus the infection takes place through scratches, abrasions or cuts in the suprabasal layers or in elective anatomical sites where basal keratinocytes are physiologically exposed, such as the squamo-columnar junction in the female genital tract or the tonsillar cryptae at the follicular, epithelial borders. In the basal cells the viral expression is largely suppressed. Nonetheless the little amount of viral proteins E5, E6 and E7 is sufficient to induce hyper-proliferation and clonal expansion of infected cells. As these cells migrate through the epithelium as a consequence of the differentiating environment the viral genome is replicated and the structural proteins L1 and L2 are formed. In the external epithelial layer these components are assembled in complete virions that are eventually shaded. Infectious competent viral progeny release is dependent on a tissue-spanning redox gradient. Both reducing and oxidant conditions are sequentially needed for infectivity maturation and redox balance modulation sharply affects the infectious titre of viral progeny [19].

The infection generally takes a chronicle course with benign epithelial proliferations eventually regressing spontaneously. Occasionally the infection can divert from this classical "productive" path, the viral infection is stably retained, the proliferation of infected cell persists indefinitely and invasive neoplasia can eventually occur. This process occurs at relevant frequencies only with a restricted number of alpha HPVs which have been accordingly named "high-risk" HPV types (HR-HPV), while the cognate "low risk" HPV term is used to indicate the non-oncogenic viral types. The carcinogenetic path represents a dead end track for the viral life cycle, as transformed cells are not permissive for virion production and no further spread of infection occurs. Accordingly cancerization is a very rare event. Nonetheless, owing to the very high prevalence of HPV infection in the general population, HPV-related cancers are highly frequent conditions representing the second neoplastic diseases among female population worldwide [20].

4. An outline of HR-HPV carcinogenic mechanisms

The oncogenic activities of HR-HPV will be hereafter outlined mostly based on data obtained about HPV-16 biological profile (Box 4), the most prevalent HR-HPV type in premalignant and malignant lesions [21] and the most intensively studied one.

The fundamental oncogenic mechanism in HPV infection is believed to be the binding of the E7 viral protein to the cellular products of the retinoblastoma (Rb) family, namely the proteins p105 (pRB), p107 and p130, that are thus targeted for proteasomal degradation [22,23]. The degradation of these factors results in the release and activation of the E2F factor that enables the expression of the "S" phase genes. In addition to, and independently from the pRb function, E7 interacts with the 600-kDa Rb protein associated factor, p600. This is a cellular factor involved in anchorage-independent growth, in integrin mediated signaling and membranes organization, whose interaction with E7 appears essential for transformation, although the precise mechanisms of such an interaction and its molecular effects remain obscure [24].

The activation of "S" phase genes, out of context in the differentiation-committed basal cell, would lead to inhibition of cell proliferation and apoptosis through a p53 dependent pathway with abortion of the viral cycle. To circumvent this cellular control and permit the completion of viral replication, the E6 protein targets the cellular p53 for ubiquitination and proteasomal degradation, resulting in suppression of apoptosis and other p53-related functions.

In the same way, and independently from p53 degradation, the E6 promotes NFX1-91 degradation. This is a repressor of the catalytic subunit of human telomerase reverse transcriptase (hTERT). As a consequence, the repression on the hTERT promoter is released and, through a complex network of downstream events including the formation of an E6/-AP/-c-myc complex, hTERT is expressed and activated permitting an extended, potentially unlimited, number of the cell replications [25–28].

Moreover, the E6 protein has the ability to interact with the extrinsic apoptosis pathway by binding to key signaling molecules, such as the Fas-associated death domain (FADD) and caspase 8 [29]. More specifically, E6 binds to the N terminus of the death effector domains of FADD and procaspase 8 [30]. This binding blocks the interaction between the two cellular proteins and increases their degradation. As a result, the activation of caspases 8, 3 and 2 is suppressed, thus allowing HPV-infected cells to avoid clearance through apoptosis [31].

In addition, the E6/AP complex protein through its E6-PDZ binding domain interacts with, and targets to the proteolytic degradation, a number of PDZ-containing proteins. Presently the actual biological role of these proteins in the viral infection/transformation is not clear, but they include potential oncosuppressor proteins, tight junction proteins, membrane associated guanylate kinases and polarity maintenance proteins. It is therefore conceivable that, independently from the p53 interaction, the binding of E6 with PDZ proteins may represent a further mechanism of de-differentiation that favors the cell migration and metastasis through the loss of cell adhesion and polarity.

On this background of unrestricted cell growth (no pRB, hTERT activation), suppression of apoptosis (no p53, caspase suppression), suppression of DNA damage sensing and repair (no p53), loss of cell adhesion and polarity (no PDZ domain proteins), the combined action of E6 and E7 further potentiates the accumulation of structural and molecular genetic damage, inducing multiple deregulations in centrosome duplication and mitotic spindle organization and function [32,33], as well as suppression of mitotic checkpoints and structural alteration of chromosomes [34–36].

The HPV transforming activity is further promoted by the E5 viral oncogene. This is a small, comparatively under-explored protein of approximately 83 amino acids. It has minor transforming activity per se; however it is able to potentiate the activity of the major oncogenes E6 and E7 through a number of mechanisms mainly acting during the early steps of viral infection/transformation. The mechanisms activated by E5 include the EGF-R mediated signal transduction and the downstream pathways of Ras–Raf–MAP kinase or PI3K–Akt. As a whole, these result in enhanced response to cell proliferative stimuli, increased activation of pro-angiogenic pathways, suppression of apoptosis, induction of antioxidant response and oxidative damage repairs [37].

Human viruses causing cancer

Virus	Type of cancer(s)	Year of first identification	References
Epstein–Barr virus (EBV)	Burkitt's lymphoma; nasopharyngeal carcinoma; lymphoproliferative disorders, some Hodgkin's disease; some non-Hodgkin's lymphoma and some gastrointestinal lymphoma	1964	M.A. Epstein et al, Lancet 15 (1964) 702–703.
Hepatitis B virus (HBV)	Some hepatocellular carcinoma	1965	B.S. Blumberg et al, JAMA 191 (1965) 541–546.
Human T-lymphotropic virus-I (HTLV-I)	Adult T cell leukaemia	1980	B.J. Poiesz et al, Proc. Natl. Acad. Sci. USA 77 (1980) 7415–7419.
High-risk human papillomaviruses (HR-HPV)	Most cervical cancers; some other anogenital and head and neck cancers	1974-1982	H. zur Hausen, Virology 384 (2009) 260-265.
Hepatitis C virus (HCV)	Some hepatocellular carcinoma and some lymphomas	1989	Q.L. Choo et al, Science 244 (1989) 359–362.
Kaposi's sarcoma herpesvirus (KSHV)	Kaposi's sarcoma, primary effusion lymphoma and some multicentric Castleman's disease	1994	Y. Chang et al, Science 265 (1994) 1865–1869.
Merkel cell polyomavirus (MCV)	Merkel cell carcinoma	2008	H. Feng et al, Science 319 (2008) 1096–1100.

Box 2. Human viruses causing cancer.

These few basic actions of the E5, E6 and E7 viral oncogenes depict a cellular landscape conducive to genetic damage accumulation and cancer development and are those generally used to summarize the oncogenic potentials of HR-HPVs. However it has to be remarked that they represent just a minor part of the many functions of these viral oncogenes. Indeed HPV proteins are highly sophisticated multifunctional devices. They are able to interact with, and thus contribute to cell transformation, a huge number of cellular functions and factors, extensively described and discussed in excellent reviews [38–40].

5. The role of oxidative stress in cervical cancer

Although HPV is undoubtedly implicated as the causative agent of cervical cancer, the mere HR-HPV infection is not sufficient for tumor development and other additional factors have to take part in the process. It has been suggested that the redox balance and the oxidative stress (OS) may play relevant roles in HPV dependent carcinogenesis, acting synergistically both at the neoplastic initiation and progression. Both epidemiological studies and biochemical data suggest that OS is a condition that favors different stages of viral infection including the viral adsorption, the viral entry and the initial establishment of viral expression [41]. It is known that enhanced OS characterizes cancer cells. Elevated rate of reactive oxygen species (ROS) production has been detected in almost all cancers, where they act as second messengers in intracellular signaling cascades, promoting many aspects of tumor development and progression [42]. Further some risk factors known to be implicated in cervical cancer development, such as tobacco smoking and chronic inflammation, cause an elevation of OS [43].

The current review discusses the possibility that HPV is able to activate several antioxidant defenses counteracting increasing ROS levels and managing its own transcriptional regulation. By this way, infected cells can aberrantly proliferate. The fascinating picture that emerges is that HPV oncogenes confer to the infected cells the ability to survive in an increasing oxidant environment, through different mechanisms:

- Regulation of antioxidant enzymes and compounds,
- Protection from oxidation of detoxifying/antiapoptotic enzymes and redox-sensitive transcription factors,
- Suppression of OS-induced apoptosis,
- Modulation of host microRNAs regulating genes associated with antioxidant defense.

In the section below, experimental evidences addressing the outlined mechanisms are discussed.

6. Regulation of antioxidant enzymes and compounds

6.1. Catalase

Studies performed on HaCaT keratinocyte cell line showed that cells stably expressing the E7 were more resistant to hydrogen peroxide-induced toxicity than parental cells [44], showing a considerably reduced cell death. Such an increased resistance was coupled with reduced intracellular ROS and a parallel increase in the levels of catalase. Moreover, catalase expression levels increased in HaCaT/E7 cells treated with H_2O_2 in a dose-dependent manner, supporting the notion that

A relative evaluation of HPV carcinogenic potential

Condition	RR	References
Breast cancer in women undergoing HRT	1.3	Women's Health Initiative, JAMA 288 (2002) 321-333.
Lung cancer in white adult male smoker	8.3	M. Plummer et al, Cancer Causes Control 14 (2003) 805-814.
Cervical cancer in women with HPV 16 infection	434.5	N. Munoz et al, N. Engl. J. Med. 348 (2003) 518-527.

Comparing the Relative oncogenic Risk (RR) of HPV infection to the ones associated with well known risk factors for cancers at other sites gives us a plastic representation its carcinogenic power.
A very powerful reaction of the public opinion accompanied the publication of data showing that women receiving post menopausal hormone replacement therapy (HRT) had an hazards ratio of Breast Cancer of (only) 1.3.
Smoking cigarettes is a highly dangerous behavior associated with lung cancer.
In white male smokers with a 8 year lasting habit to smoke around 20 cigarettes per day the RR is about 8.3 times the one of non-smokers
Remarkably women who were positive for a persistent cervical infection by HPV 16 had an odds ratio of having invasive cervical cancer of 434 times higher than HPV 16 negative women.

Box 3. A relative evaluation of HPV carcinogenic potential.

increased resistance of E7 expressing cells occurred through the scavenging of intracellular ROS. The increased activity of catalase was the result of a dual action of the E7 expression, which enhanced both the activities of catalase promoter and NF-kB, one of the major transcription factors regulating the expression of the catalase gene. These effects were E7 specific, as shown by transient expression of an E7 antisense vector. In a following study, the same group [45] investigated the effect of E7 oncogene on the receptor mediated apoptosis, cell death via the mitochondrial pathway and modulation of apoptosis related factors, showing that the E7 expression, in addition to catalase, also modulated the expression of Bcl-xL, IL-18, Fas, Bad, and cytochrome c as well as NF-kB, resulting in a multifactorial resistance to OS-induced cell death.

The upregulation of catalase following the expression of E7 in HaCaT cells was also confirmed by proteomics analyses [46] which found catalase among a group of 28 modulated proteins.

6.2. SOD and NAD(P)H:quinone oxidoreductase 1 (NQO1)

During studies with HK-168 cells, a cell line derived from normal human epithelial keratinocytes "in vitro" transformed with the whole HPV-16 genome, another potentiation of OS detoxification mechanisms was reported [47]. Following UVB-irradiation only a mild apoptotic effect was observed in HK-168 cells, while HaCaT cells, devoid of viral genes, consistently showed a much deeper cell death. In HK-168, the mild reduction of cell proliferation was accompanied by a sharp elevation of SOD and NQO1 activity, a detoxifying enzyme mainly involved in lipid and protein quinone detoxication.

6.3. Peroxiredoxins (Prxs)

These cysteine-rich proteins are members of the antioxidant defense network. They participate to the regulation of redox balance by removing H_2O_2, are involved in signal transduction pathways in response to both physiological and oxidative stimuli and play a fundamental role in both carcinogenesis and tumor progression [48]. It has been shown that overexpression of Prxs prevented the production of ROS [49] and various Prx isoforms have been found overexpressed in several types of tumors [50]. The above-cited works from Shim et al. [44,45] showed the up-regulation of Prx 1 and 2 expression in E7-expressing HaCaT cells and Prx 2 elevation was also reported in the proteomic study of Lee et al. [46]. About cervical cancer, the study by Kim et al. [51] showed a direct correlation between Prx 2 and 3 expression and severity of cervical intraepithelial neoplasiae (CIN) (the precancerous cervical cancer lesion) at any grade. Recently Hu et al. [52] reported, in histological samples from invasive cervical carcinomas, an increased expression of Prx3. This protein was highly expressed in cancer areas while only a slight positivity was observed in the basal layer of adjacent non-neoplastic tissue. Moreover, the pattern of Prx3 expression in cervical cancer cells was consistent with that of Ki67, a cell proliferation-associated nuclear protein expressed in the G1, S, G2 and M phases of the cell cycle, suggesting that Prx3 is a potential marker for tumor proliferation.

7. Protection from oxidation

7.1. Detoxifying enzymes

The genes belonging to the glutathione S-transferase (GST) family encode enzymes that appear to be critical in cellular protection against the cytotoxic effects of stress conditions. GSTs play an important role in conjugating GSH to the products of endogenous lipid peroxidation and inactivating organic hydroperoxides formed as secondary metabolites during OS [53,54]. GSTs were found to be overexpressed in different tumors and their increased expression was associated with multidrug resistance and a worse clinical prognosis [55,56]. Polymorphisms of some GST (GSTM1 and GSTT1) family members have been associated to the risk of developing squamous cervical carcinoma [57]. Accordingly, a study from our group [58] on HPV-16 positive tissues collected from patients with invasive squamous cervical carcinoma or with high grade dysplastic HPV lesions showed increased GST expression levels in dysplasia and even more in cancer specimens compared with control tissue.

Cytosolic GSTs have a dimeric structure due to a noncovalent association of identical or different subunits with a molecular mass of 23–28 kDa [59]. Moreover, each monomer contains one binding site for

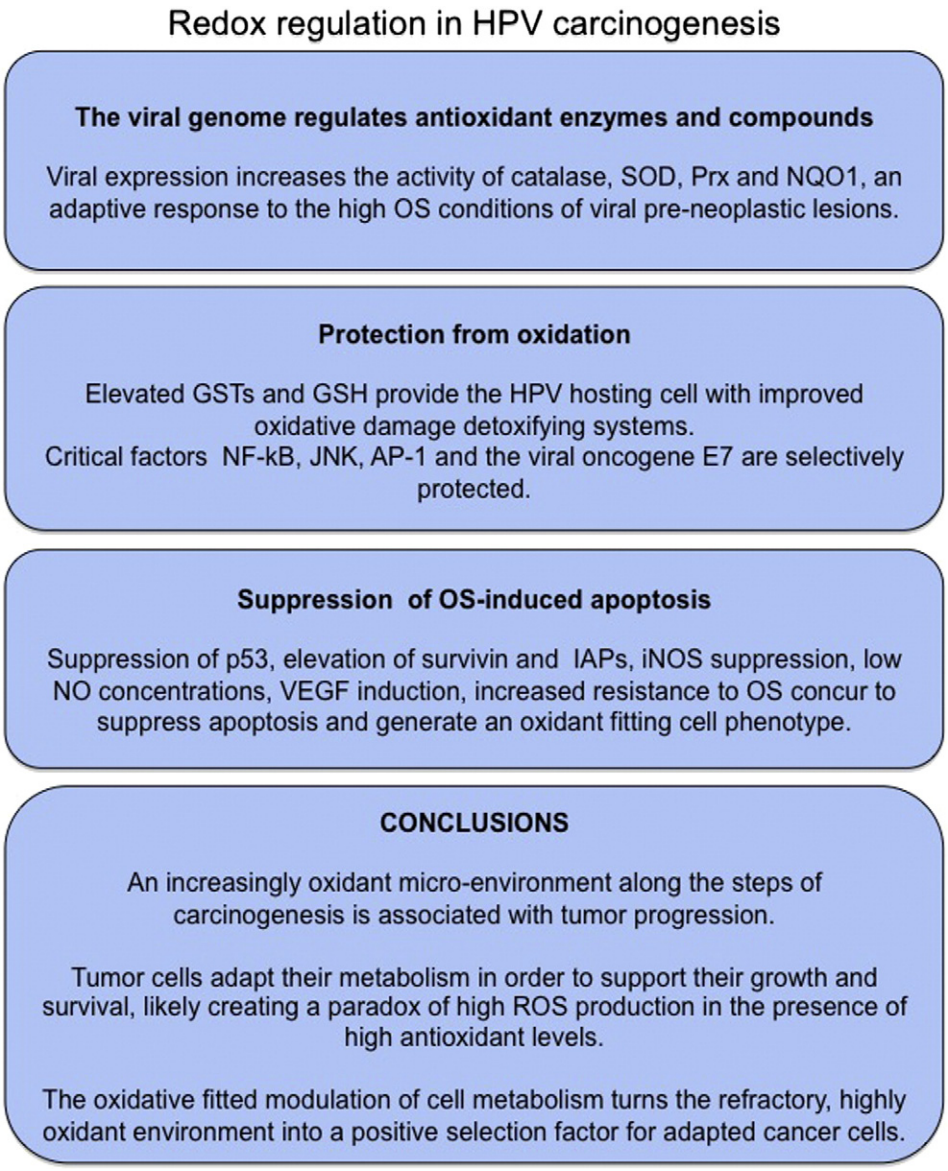
HPV at a glance

Human papillomaviruses are the etiologic agents of cervical cancer, the second cause of neoplastic death in women worldwide.
Viral infection is mostly a self-limiting spontaneously resolving condition.
Cancer is a very rare consequence of viral infection.
Expression of viral genome is necessary but is not sufficient for cancer development. Other concurring factors are needed.
Three oncogenes named E5, E6 and E7 are invariably found in High Risk HPVs (HR-HPV).
The E7 suppresses the Rb family of proteins permitting the cell to re-activate the S phase genes and interacts with the p600, having a direct trasforming activity.
The E6 suppresses the p53, promotes the transcription of hTERT, abrogates the extrinsic apoptosis pathway and suppresses a number of PDZ-domain proteins.
E5 activates the EGF-R mediated signal transduction and the downstream pathways of Ras-Raf-MAP Kinase or PI3K-Akt and promotes angiogenesis.
The coordinated expression of E5, E6 and E7 induces chromosomal aberrations, aberrant centriole proliferation, multipolar mitotic spindle and abrogation of several mitotic checkpoints.

Box 4. HPV at a glance.

glutathione and another for the hydrophobic substrate [60]. Cysteine 47, which is the residue with the highest reactivity towards thiol-specific reagents, has a crucial role. In fact, the redox modification of this residue is incompatible with the catalytic activity of the enzyme. By oxidative attack disulfide bonds can be generated, either intramolecularly between cysteines 47 and 101 in each subunit, either intermolecularly, generating multimeric forms. The redox process involving cysteine 47 leads to both inactivation and structural change of the enzyme [60].

In a screening for the host cell proteins that interact with recombinant HPV-16 E7, using a HPV-16 E7 construct with subsequent identification by mass spectrometry, glutathione S-transferase P1-1 (GSTP1) was identified as one of the major partners of HPV-16 E7 [61]. The authors showed that the amount of multimeric oxidized GSTP1 was reduced in HPV-16 E7-expressing cells compared with control cells, even more after UV irradiation or exposure to H_2O_2, which caused a substantial increase in the oxidized forms of the enzyme in control cells. A direct link between the HPV-16 E7 viral factor and the GSTP1 protein has been proposed. HPV-16 E7 binds to GSTP1 through a region that comprises amino acids 40–60 of the E7 sequence, and the E7-GSTP1 interaction modifies the redox equilibrium between the reduced and oxidized GSTP1 protein in favor of the reduced state of the enzyme. By utilizing a model in which HPV-16 E7 occupies the space between Cys 47 and Cys 101 of GSTP1 and prevents both intramolecular and intermolecular disulfide formation, the authors propose that HPV-16 E7 binding might protect GSTP1 against inactivation via oxidative attacks at cysteine residues. It is reasonable to hypothesize that the role of HPV-16 E7 is to establish a subset of GSTP1 molecules that is inaccessible to oxidative attack, thus creating a reservoir of reduced GSTP1. Stabilization of the enzyme in its active reduced form allows detoxification from OS by-products and accounts for its anti apoptotic JNK-dependent properties [62,63]. In fact, the reduced form of GSTP1 interacts and inhibits the c-Jun N-terminal kinase (JNK), negatively regulating its ability to phosphorylate the Jun protein. Since JNK-mediated signal transduction leads to apoptosis, this can be a mechanism by which HPV16 E7 transformed keratinocytes escape apoptosis.



Box 5. Redox regulation in HPV carcinogenesis.

7.2. Growth factors

The HPV transforming activity is also dependent on the availability of a defined set of transcription factors derived from the infected host cell. It is well established that gene expression is significantly regulated by ROS-mediated signaling and requires the activation of specific redox-sensitive transcription factors, necessary to ensure cell survival. It is likely that low levels of ROS/RNS are able to induce the expression of transcription factors while high levels inhibit viral transcription through downregulation of redox-sensitive transcription factors. An example is the activator protein-1 (AP-1), which closely regulates proliferation and transformation of tumor cells. Antinore et al. [64] showed that E7 complexes with AP-1 transcription factors, including c-Jun, JunB, JunD and c-Fos. AP-1 activation leads to the induction of JNK activity resulting in the phosphorylation of the c-Jun transactivation domain. On the contrary, high concentrations of ROS/RNS inhibited AP-1 and AP-1-induced gene expression. The inhibition of AP-1/DNA interactions is caused by the oxidation of specific cysteine residues in c-Jun's DNA binding region. It has been reported that the DNA binding affinity of AP-1, as well as the expression of its constituent members, varies as a function of the severity of cervical lesions [65]. In fact, while AP-1 binding was very low or absent in normal as well as in premalignant lesions, AP-1 transcription and binding activity were found to be very high in malignant tissues. It was also shown that antioxidant-induced changes of the AP-1 transcription complex were paralleled by a selective suppression of HPV transcription [66].

Similarly, NF-κB contains a redox-sensitive critical cysteine residue that is involved in DNA binding [67]. NF-κB is normally sequestered in the cytoplasm by IκB, but under oxidative conditions IκB is phosphorylated by IκB kinase (IKK), ubiquitinated, and subsequently degraded. ROS production appears to be necessary to initiate the events leading to the dissociation of the NF-κB/IκB complex [68] but excessive ROS production results in the oxidation of cysteine residue which does not affect its translocation to the nucleus, but rather interferes with DNA binding and decreases gene expression [69].

7.3. E7 oncogene

It is interesting to underline that HPV is able to protect its own oncogenes from oxidation. In fact, despite the presence of a pro-oxidant

environment in HPV-transformed tissue, some of the cysteine residues of E7 are maintained in their reduced functional status. On the basis of its high cysteine content, HPV16 E7 can be considered as a cysteine-rich protein, with seven out of 98 residues (7%) corresponding to this amino acid, a proportion well above the overall average content for mammalian proteins (2.3%). All E7 proteins from high-risk types contain at least two non-canonical cysteines in their sequence. It has been demonstrated that, despite its high cysteine content, all seven cysteine residues within HPV16 E7 are in their reduced state under conditions that resemble the basal reducing environment of the cell cytoplasm [70]. However, E7 undergoes a controlled oxidation process upon the addition of biologically compatible oxidants, such as H_2O_2 and oxidized glutathione. By using a combination of protein mutagenesis, spectroscopy, and mass spectrometry techniques, Chemes et al. [70] described two distinct redox centers in the HPV16 E7 oncoprotein involving the cysteine 24 and the cysteine 59/68 pair and demonstrated the protective effect of non-canonical cysteine 59 on the overall E7 redox state. Cysteine 24 in the HPV16 E7 sequence is located within a short linear motif responsible for Rb binding. The redox status of this cysteine is therefore fundamental for the Rb binding. The reported results showed that, in the context of the full-length protein, cysteine 24 is protected from oxidation and less prone to glutathionylation than in the context of the isolated N terminal domain, indicating that the C-terminal cysteine-rich domain can prevent the GSSG-induced oxidation of cysteine 24. Considering that the E7 oncoprotein performs its functions in an oxidative environment, a reasonable hypothesis is that non-canonical cysteine residues protect the E7 protein against oxidative damage and consequent loss of function. Therefore, maintaining Cys 24 in a reduced state allows Rb binding despite the presence of the oxidative environment observed in HPV-transformed tissues.

8. Suppression of OS-induced apoptosis

Resistance of HPV-infected cells to programmed cell death induced by oxidant conditions is achieved by regulation of some apoptosis inhibitors:

8.1. Survivin

Survivin is a member of the inhibitor of apoptosis protein (IAP) family. Besides its capacity to suppress apoptosis and control cell division, survivin has been shown to act as an antioxidant compound. Baratchi et al. [71] demonstrated that survivin increases the antioxidant activity of GST, SOD, catalase and GSH-reductase, and effectively counteracts oxidant activity following exposure to H_2O_2. Further, a recent study from Kan et al. [72] indicated that the up-regulation of survivin confers non-transformed immortal cell resistance to OS, a condition that favors the early stages of carcinogenesis. Survivin is selectively expressed in tumor cells and is present in most malignant tumors, including cervical cancer [73]. HPV-16 E6, but not E7, was found to significantly transactivate the survivin promoter [74]. Experiments performed in different cancer cell lines and with different E6 mutants indicated that the effect of E6 on the survivin promoter is largely dependent on p53 status. Accordingly, the p53 tumor suppressor protein down-regulated the expression of survivin. Considering that E6 is able to interact with p53 and induces its ubiquitin-dependent degradation, it appears that the transactivation effect of E6 on survivin is mediated by the p53 degradation pathway.

8.2. c-IAP2 (cellular inhibitor of apoptosis protein-2)

IAPs are anti-apoptotic proteins, well known for their ability to inhibit caspase activation and permit cell survival in stress conditions. From this standpoint, they are attractive targets for facilitating apoptosis in cancer cells. It has been shown in various cell types that up-regulation of cIAP-2 expression confers resistance to OS-mediated apoptosis [75]. In E6- and E7-expressing oral keratinocytes, as well as in cancer-derived HPV-positive cell lines, it was shown that both oncogenes induced cIAP-2 expression and that its depletion in these cells led to apoptosis [76]. Among proposed mechanisms, NF-κB mediated signaling cascade seems to play a role. In fact, the cIAP induction was found to require NF-κB activity. It has been proved that E6-induced expression of cIAP-2 was directly associated with the oncogene ability to induce NF-κB binding to the cIAP-2 promoter, thus increasing cIAP-2 transcript level [77]. Recently, evidences were provided about a mechanism for transcriptional regulation of cIAP-2 involving ERK1/2, IκBα phosphorylation and degradation and consequent NF-κB activation [78].

8.3. i-NOS

Nitric oxide (NO), synthesized by the nitric oxide synthase (NOS), is a bioactive molecule that mediates a number of actions including neurotransmission, vasculature modulation, parasites killing and antineoplastic activity [79]. A large body of evidence indicates that NO might have different effects depending on the local concentration of the molecule. Low concentration of NO promotes tumor growth and angiogenesis whereas at high concentration NO has anti-tumor activity by inducing cytotoxicity and apoptosis [80]. The inducible NOS isoform (iNOS) is a well-established marker of nitrosative stress and inflammation; it can be induced in a variety of cell types and produces high concentrations of NO. However, in the case of cervical carcinomas and HPV-positive dysplastic lesions, NO fails to attain apoptosis-inducting concentration and the reason for the inadequate level of NO is the insufficient expression of iNOS that appears to be progressively reduced with the histological severity of lesions [58,81]. This is the result of the TGFβ-1-mediated suppression of the iNOS expression at the mRNA level [82]. Accordingly, the level of TGFβ-1 mRNA observed in cervical smears correlates with the progression of cervical intraepithelial neoplasia to cancer [83].

Meanwhile, low level of NO promotes mutagenesis, that further accelerates the neoplastic progression. E6 protein, in a p53-independent way, specifically upregulates the activity of the VEGF promoter, thereby inducing a high level of VEGF mRNA expression, so promoting the VEGF mediated angiogenesis, that provides optimal metabolite supply to the tumor growth. Moreover, the increased blood flow potentiates the NO scavenging by circulating red blood cells [84] thus completely arresting tumor apoptosis.

9. Modulation of host microRNAs: miR 34a

In the recent years, several studies have focused on the role of microRNAs (miRNAs) in the development of cervical cancer. The miRNAs are short non-coding regulatory RNAs that control gene expression at the post-transcriptional level, through inhibition of transcription or degradation of definite canonical mRNAs. Deregulation of miRNAs expression has been discovered in a wide variety of tumors and it is now clear that they contribute to cancer development and progression [85–88]. Regulation of cellular miRNA expression by HPV has been extensively reviewed by Zheng and Wang [89] which report a summary of miRNA expression profiling studies in cervical cancer. An aberrant expression profile of several miRNAs is evident, which can be correlated with the activity of E6 and E7 oncoproteins. A list of miRNAs involved in the development and progression of cervical cancer is available in the review of Pedroza-Torres et al. [90]. Many miRNAs are associated with cervical cancer, either upregulated or downregulated. For instance, a frequent loss of miRNA-218 (miR-218) was reported [91]. While miR-218 overexpression induced cellular apoptosis and suppressed tumor growth [92], its loss was associated with tumor progression and poor prognosis.

The miR-34 family of miRNAs has emerged as important components of p53-mediated responses [93] by targeting substrates involved in cell cycle regulation and apoptosis [94]. miR-34a is tumor suppressive, that exerts antiproliferative effects and contributes to p53-

mediated apoptosis [95]. Genes encoding miRNAs in the miR-34 family are direct transcriptional targets of p53 [94,96] and both miR-34a transcription and expression were shown directly stimulated by p53 [96]. In cervical cancer, the p53 suppression operated by the E6 oncoprotein downregulates the expression of miR-34a, as reported by several authors [90,97,98].

The miR-34a suppression leads as final consequence to apoptosis inhibition and ROS decrease, through different ways (Fig. 1):

a) Among the targets of miR-34a there is the suppression of thioredoxin reductase 2 (Trxrd2) [99]. The Trx system contributes to the regulation of the intracellular redox status and redox signaling cascades, catalyzes the reduction of a wide range of substrates, is essential for the carcinogenic process and cancer invasivity [100,101] and modulates sulfhydryl–disulfide isomerization reactions governing the conformation and function of p53, as well as several other redox sensitive transcription factors [102,103]. Thus, during HPV infection viral oncogene E6 determines the degradation of p53; consequently, miR34a is suppressed, and Trxrd2 is not inhibited.
b) Another mechanism implicates uncoupling protein 2 (UCP2), a negative regulator of ROS generation [104]. In fact, it was shown that the silencing of the UCP2 gene led to increased rate of ROS generation and UCP2 ablation determined a marked increase of OS in several cell types [105]. miR-34a has been indicated to target the gene of UCP2 [106] and both UCP2 gene and protein levels were downregulated coupled with differential expression of miR-24 and -34a [107].
c) Sirtuin 1 (SIRT1) expression is downregulated by the miR-34a signaling pathway and the activation of this latter plays important roles in enhancing cell sensitivity to OS-induced apoptosis [108]. Accordingly, suppression of SIRT1 expression by miR-34a was shown to reinforce p53 activation [109,110] and restore apoptotic sensitivity [111]. During HPV infection, as a consequence of the E6-induced inactivation of p53 that determines downregulation of miR 34a, SIRT1 is not repressed and is able to exert its multiple functions. SIRT1 deacetylates several transcription factors, such as p53, forkheadbox type O (FOXO) proteins, NF-kB, and coactivators which control the transcription of pro-antioxidant enzymes [112]. It has been shown that SIRT1 protects from OS toxicity, via deacetylation and activation of the FOXO3 transcription factor, that in turn leads to the induction of SOD2 expression [113,114]. Further, an increased expression of catalase resulting from deacetylation of FOXO3 caused by SIRT1 overexpression has been showed [115]. It is important to underline that SIRT1, due to its de-acetylating properties, exhibits also an anti-apoptotic effect, regulating transcriptional activity of p53. In fact, activated p53 is acetylated by CBP/p300 acetyltransferases at some lysine residues and it has been shown that acetylation of p53 at Lys 382 is inhibited via SIRT1, thus inhibiting the pro-apoptotic function of p53 [116].

10. Conclusions

HPV infection has been identified as the major etiological factor in cervical carcinogenesis. Cervical cancer is a slow-evolving disease, arising from dysplastic lesions after long persistent infection. HPV-driven carcinogenesis is a multi-step process, in which progressive histologic and cytologic changes occur, that can be classified in early lesions, currently indicated as cervical intraepithelial neoplasia 1 (CIN 1) or low grade squamous intraepithelial lesion (LSIL) and high grade lesions, known as cervical intraepithelial neoplasia 2 and 3 (CIN 2 and CIN3) or high grade squamous intraepithelial lesion (HSIL).

The HPV infection, although necessary, is not sufficient to cause cancer and several studies have been devoted to the search for concurrent carcinogenic factors. Among these co-factors, many evidences support the role of OS. It is clear that viral infection induces OS that in turn causes damage to all types of biological macromolecules. As a matter of fact, the majority of HPV infections (80%) is subclinical and transitory and is resolved spontaneously. It is reasonable to hypothesize that, while in most cases the cells react to HPV infection and can overcome the virus-induced OS by activating apoptosis leading to termination of viral replication and lesion regression, in some of the infected cells a steady state balance between OS generation and detoxification is established, partly due to viral induced antioxidant response. Thus infected cells can aberrantly proliferate, paving the way to neoplastic progression.

In this review we have discussed the possible mechanisms by which HPV exploits host cell survival mechanisms, through modulation of redox homeostasis (Box 5). Thus, tumor cells adapt their metabolism in order to support their growth and survival, likely creating a paradox of high ROS production in the presence of high antioxidant levels, to fit well with stress conditions.

This tangled scenario indicates that, although an increasingly oxidant micro-environment along the steps of carcinogenesis is associated with tumor progression, tumor cells are characterized by a rather efficient control on ROS generation and oxidative damage, achieved through the upregulation of antioxidant enzymes and detoxifying/pro-survival proteins. This oxidative fitted modulation of cell metabolism turns the refractory, highly oxidant tumor environment into a positive selection factor for adapted cancer cells.

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References

[1] P.C. Norwell, The clonal evolution of tumour cell population, Science 194 (1976) 23–28.
[2] D. Hanahan, A.R. Wienberg, The halmark of cancer, Cell 7 (2000) 57–70.
[3] V. Ellerman, O. Bang, Experimentelle leukämie bei hühnern, Centralbl. F. Bakteriol. 46 (1908) 595–609.
[4] P. Rous, A sarcoma of the fowl transmissible by an agent separable from the tumor cells, J. Exp. Med. 13 (1911) 397–411.
[5] P.S. Moore, Y. Chang, Why do viruses cause cancer? Highlights of the first century of human tumour virology, Nat. Rev. Cancer 10 (2010) 878–889.
[6] H. zur Hausen, Papillomaviruses and cancer: from basic studies to clinical application, Nat. Rev. Cancer 2 (2002) 342–350.
[7] D.M. Parkin, F. Bray, Chapter 2: the burden of HPV-related cancers, Vaccine 24 (Suppl. 3:S3) (2006) 11–25.
[8] Z.M. Zheng, C.C. Baker, Papillomavirus genome structure, expression, and post-transcriptional regulation, Front. Biosci. 11 (2006) 2286–2302.
[9] T.M. Wise-Draper, S.I. Wells, Papillomavirus E6 and E7 proteins and their cellular targets, Front. Biosci. 13 (2008) 1003–1017.
[10] S. Liao, D. Deng, W. Zhang, X. Hu, W. Wang, H. Wang, Y. Lu, S. Wang, L. Meng, D. Ma, Human papillomavirus 16/18 E5 promotes cervical cancer cell proliferation, migration and invasion in vitro and accelerates tumor growth in vivo, Oncol. Rep. 29 (2013) 95–102.
[11] J. Doorbar, Molecular biology of human papillomavirus infection and cervical cancer, Clin. Sci. (Lond.) 110 (2006) 525–541.
[12] Y. Modis, B.L. Trus, S.C. Harrison, Atomic model of the papilloma virus capsid, EMBO J. 21 (2002) 4754–4762.
[13] M. Bergvall, T. Melendy, J. Archambault, The E1 proteins, Virology 445 (2013) 35–56.
[14] A.A. McBride, The papillomavirus E2 proteins, Virology 445 (2013) 57–79.
[15] S. García-Vallvé, A. Alonso, I.G. Bravo, Papillomaviruses: different genes have different histories, Trends Microbiol. 13 (2005) 514–521.
[16] H.U. Bernard, R.D. Burk, Z. Chen, K. van Doorslaer, H. Zur Hausen, E.M. De Villiers, Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments, Virology 401 (2010) 70–79.
[17] E.M. de Villiers, C. Fauquet, T.R. Broker, H.U. Bernard, H. zur Hausen, Classification of papillomaviruses, Virology 324 (2004) 17–27.
[18] J. Doorbar, W. Quint, L. Banks, I.G. Bravo, M. Stoler, T.R. Broker, M.A. Stanley, The biology and life-cycle of human papillomaviruses, Vaccine 30 (2012) 55–70.
[19] M.J. Conway, S. Alam, E.J. Ryndock, L. Cruz, N.D. Christensen, R.B. Roden, C. Meyers, Tissue-spanning redox gradient-dependent assembly of native human papillomavirus type 16 virions, J. Virol. 83 (2009) 10515–10526.
[20] K.P. Braaten, M.R. Laufer, Human papillomavirus (HPV), HPV-related disease, and the HPV vaccine, Rev. Obstet. Gynecol. 1 (2008) 2–10.
[21] N. Munoz, F.X. Bosch, S. de Sanjose, R. Herrero, X. Castellsague, K.V. Shah, P.J. Snijders, C.J. Meijer, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group, Epidemiologic classification of human papillomavirus types associated with cervical cancer, N. Engl. J. Med. 348 (2003) 518–527.

[22] N. Dyson, P.M. Howley, K. Münger, E. Harlow, The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product, Science 243 (1989) 934–937.
[23] K. Münger, J.R. Basile, S. Duensing, A. Eichten, S.L. Gonzalez, M. Grace, V.L. Zacny, Biological activities and molecular targets of the human papillomavirus E7 oncoprotein, Oncogene 20 (2001) 7888–7898.
[24] K.W. Huh, J. DeMasi, H. Ogawa, Y. Nakatani, P.M. Howley, K. Münger, Association of the human papillomavirus type 16 E7 oncoprotein with the 600-kDa retinoblastoma protein-associated factor, p600, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11492–11497.
[25] H.R. McMurray, D.J. McCance, Human papillomavirus type 16 E6 activates TERT gene transcription through induction of c-Myc and release of USFmediated repression, J. Virol. 77 (2003) 9852–9861.
[26] T. Veldman, X. Liu, H. Yuan, R. Schlegel, Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8211–8216.
[27] L. Gewin, H. Myers, T. Kiyono, D.A. Galloway, Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex, Genes Dev. 18 (2004) 2269–2282.
[28] R.A. Katzenellenbogen, E.M. Egelkrout, P. Vliet-Gregg, L.C. Gewin, P.R. Gafken, D.A. Galloway, NFX1-123 and poly(A) binding proteins synergistically augment activation of telomerase in human papillomavirus type 16 E6-expressing cells, J. Virol. 81 (2007) 3786–3796.
[29] M. Filippova, M.M. Johnson, M. Bautista, V. Filippov, N. Fodor, S.S. Tungteakkhun, K. Williams, P.J. Duerksen-Hughes, The large and small isoforms of human papillomavirus type 16 E6 bind to and differentially affect procaspase 8 stability and activity, J. Virol. 81 (2007) 4116–4129.
[30] S.S. Tungteakkhus, M. Filippova, J.W. Neidigh, N. Fodor, P.J. Duerksen-Hughes, The interaction between human papillomavirus type 16 and FADD is mediated by a novel E6 binding domain, J. Virol. 82 (2008) 9600–9614.
[31] T. Garnett, M. Filippova, P.J. Duerksen-Hughes, Accelerated degradation of FADD and procaspase 8 in cells expressing human papilloma virus 16 E6 impairs TRAIL-mediated apoptosis, Cell Death Differ. 13 (2006) 1915–1926.
[32] S. Duensing, L.Y. Lee, A. Duensing, J. Basile, S. Piboonniyom, S. Gonzalez, C.P. Crum, K. Münger, The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10002–10007.
[33] A. Duensing, Y. Liu, M. Tseng, M. Malumbres, M. Barbacid, S. Duensing, Cyclin-dependent kinase 2 is dispensable for normal centrosome duplication but required for oncogene-induced centrosome overduplication, Oncogene 25 (2006) 2943–2949.
[34] S. Duensing, K. Münger, The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability, Cancer Res. 62 (2002) 7075–7082.
[35] C. Vogel, A. Kienitz, I. Hofmann, R. Müller, H. Bastians, Crosstalk of the mitotic spindle assembly checkpoint with p53 to prevent polyploidy, Oncogene 23 (2004) 6845–6853.
[36] Y. Liu, S.A. Heilman, D. Illanes, G. Sluder, J.J. Chen, P53-independent abrogation of a postmitotic checkpoint contributes to human papillomavirus E6-induced polyploidy, Cancer Res. 67 (2007) 2603–2610.
[37] A. Venuti, F. Paolini, L. Nasir, A. Corteggio, S. Roperto, M.S. Campo, G. Borzacchiello, Papillomavirus E5: the smallest oncoprotein with many functions, Mol. Cancer 10 (2011) 140.
[38] M.A. Stanley, M.R. Pett, N. Coleman, HPV: from infection to cancer, Biochem. Soc. Trans. 35 (2007) 1456–1460.
[39] T. Yugawa, T. Kiyono, Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: novel functions of E6 and E7 oncoproteins, Med. Virol. 19 (2009) 97–113.
[40] C.A. Moody, L.A. Laimins, Human papillomavirus oncoproteins: pathways to transformation, Nat. Rev. Cancer 10 (2010) 550–560.
[41] V.M. Williams, M. Filippova, U. Soto, P.J. Duerksen-Hughes, HPV–DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress, Futur. Virol. 6 (2011) 45–57.
[42] G.Y. Liou, P. Storz, Reactive oxygen species in cancer, Free Radic. Res. 44 (2010) 479–496.
[43] A. Moktar, R. Singh, M.V. Vadhanam, S. Ravoori, J.W. Lillard, C.G. Gairola, R.C. Gupta, Cigarette smoke condensate-induced oxidative DNA damage and its removal in human cervical cancer cells, Int. J. Oncol. 39 (2011) 941–947.
[44] J.H. Shim, K.J. Cho, K.A. Lee, S.H. Kim, P.K. Myung, Y.K. Choe, D.Y. Yoon, E7-expressing HaCaT keratinocyte cells are resistant to oxidative stress-induced cell death via the induction of catalase, Proteomics 5 (2005) 2112–2122.
[45] J.H. Shim, K.H. Kim, Y.S. Cho, H.S. Choi, E.Y. Song, P.K. Myung, J.S. Kang, S.K. Suh, S.N. Park, D.Y. Yoon, Protective effect of oxidative stress in HaCaT keratinocytes expressing E7 oncogene, Amino Acids 34 (2008) 135–141.
[46] K.A. Lee, J.W. Kang, J.H. Shim, C.W. Kho, S.G. Park, H.G. Lee, S.G. Paik, J.S. Lim, D.Y. Yoon, Protein profiling and identification of modulators regulated by human papillomavirus 16 E7 oncogene in HaCaT keratinocytes by proteomics, Gynecol. Oncol. 99 (2005) 142–152.
[47] F. De Marco, M. Perluigi, C. Foppoli, C. Blarzino, C. Cini, R. Coccia, A. Venuti, UVB irradiation down-regulates HPV-16 RNA expression: implications for malignant progression of transformed cells, Virus Res. 130 (2007) 249–259.
[48] L.H. Butterfield, A. Merino, S.H. Golub, H. Shau, From cytoprotection to tumor suppression: the multifactorial role of peroxiredoxins, Antioxid. Redox Signal. 1 (1999) 385–402.
[49] C.M. Wong, A.C. Chun, K.H. Kok, Y. Zhou, P.C. Fung, H.F. Kung, K.T. Jeang, D.Y. Jin, Characterization of human and mouse peroxiredoxin IV: evidence for inhibition by Prx-IV of epidermal growth factor- and p53-induced reactive oxygen species, Antioxid. Redox Signal. 2 (2000) 507–518.
[50] B. Zhang, Y. Wang, Y. Su, Peroxiredoxins, a novel target in cancer radiotherapy, Cancer Lett. 286 (2009) 154–160.
[51] K. Kim, M. Yu, S. Han, I. Oh, Y.J. Choi, S. Kim, K. Yoon, M. Jung, W. Choe, Expression of human peroxiredoxin isoforms in response to cervical carcinogenesis, Oncol. Rep. 21 (2009) 1391–1396.
[52] J.X. Hu, Q. Gao, Peroxiredoxin 3 is a novel marker for cell proliferation in cervical cancer, Biomed. Rep. 1 (2013) 228–230.
[53] J.D. Hayes, D.J. Pulford, The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance, Crit. Rev. Biochem. Mol. Biol. 30 (1995) 445–600.
[54] J.D. Hayes, J.U. Flanagan, I.R. Jowsey, Glutathione transferases, Annu. Rev. Pharmacol. Toxicol. 45 (2005) 51–88.
[55] D.T. Dang, F. Chen, M. Kohli, C. Rago, J.M. Cummins, L.H. Dang, Glutathione S-transferase pi1 promotes tumorigenicity in HCT116 human colon cancer cells, Cancer Res. 65 (2005) 9485–9494.
[56] C.C. McIlwain, D.M. Townsend, K.D. Tew, Glutathione S-transferase polymorphisms: cancer incidence and therapy, Oncogene 25 (2006) 1639–1648.
[57] Y. Liu, L.Z. Xu, Meta-analysis of association between GSTM1 gene polymorphism and cervical cancer, Asian Pac. J. Trop. Med. 5 (2012) 480–484.
[58] F. De Marco, E. Bucaj, C. Foppoli, A. Fiorini, C. Blarzino, K. Filipi, A. Giorgi, M.E. Schininà, F. Di Domenico, R. Coccia, D.A. Butterfield, M. Perluigi, Oxidative stress in HPV-driven viral carcinogenesis: redox proteomics analysis of HPV-16 dysplastic and neoplastic tissues, PLoS ONE 7 (2012) e34366.
[59] B. Wu, D. Dong, Human cytosolic glutathione transferases: structure, function, and drug discovery, Trends Pharmacol. Sci. 33 (2012) 656–668.
[60] G. Ricci, G. Del Boccio, A. Pennelli, M. Lo Bello, R. Petruzzelli, A.M. Caccuri, D. Barra, G. Federici, Redox forms of human placenta glutathione transferase, J. Biol. Chem. 266 (1991) 21409–21415.
[61] A.M. Mileo, C. Abbruzzese, S. Mattarocci, E. Bellacchio, P. Pisano, A. Federico, V. Maresca, M. Picardo, A. Giorgi, B. Maras, M.E. Schininà, M.G. Paggi, Human papillomavirus-16 E7 interacts with glutathione S-transferase P1 and enhances its role in cell survival, PLoS ONE 4 (2009) e7254.
[62] T. Wang, P. Arifoglu, Z. Ronai, K.D. Tew, Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus, J. Biol. Chem. 276 (2001) 20999–21003.
[63] V. Adler, Z. Yin, S.Y. Fuchs, M. Benezra, L. Rosario, K.D. Tew, M.R. Pincus, M. Sardana, C.J. Henderson, C.R. Wolf, R.J. Davis, Z. Ronai, Regulation of JNK signaling by GSTp, EMBO J. 18 (1999) 1321–1334.
[64] M.J. Antinore, M.J. Birrer, D. Patel, L. Nader, D.J. McCance, The human papillomavirus type 16 E7 gene product interacts with and trans-activates the AP1 family of transcription factors, EMBO J. 15 (1996) 1950–1960.
[65] B.K. Prusty, B.C. Das, Constitutive activation of transcription factor AP-1 in cervical cancer and suppression of human papillomavirus (HPV) transcription and AP-1 activity in HeLa cells by curcumin, Int. J. Cancer 113 (2005) 951–960.
[66] F. Rösl, B.C. Das, M. Lengert, K. Geletneky, H. zur Hausen, Antioxidant-induced changes of the AP-1 transcription complex are paralleled by a selective suppression of human papillomavirus transcription, J. Virol. 71 (1997) 362–370.
[67] J.R. Matthews, W. Kaszubska, G. Turcatti, T.N. Wells, R.T. Hay, Role of cysteine62 in DNA recognition by the P50 subunit of NF-kappa B, Nucleic Acids Res. 21 (1993) 1727–1734.
[68] J.M. Hansen, Y.M. Go, D.P. Jones, Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling, Annu. Rev. Pharmacol. Toxicol. 46 (2006) 215–234.
[69] S. Nair, W. Li, A.N. Kong, Natural dietary anti-cancer chemopreventive compounds: redox-mediated differential signaling mechanisms in cytoprotection of normal cells versus cytotoxicity in tumor cells, Acta Pharmacol. Sin. 28 (2007) 459–472.
[70] L.B. Chemes, G. Camporeale, I.E. Sánchez IE, G. de Prat-Gay, L.G. Alonso, Cysteine-rich positions outside the structural zinc motif of human papillomavirus E7 provide conformational modulation and suggest functional redox roles, Biochemistry 53 (2014) 1680–1696.
[71] S. Baratchi, R.K. Kanwar, J.R. Kanwar, Survivin mutant protects differentiated dopaminergic SK-N-SH cells against oxidative stress, PLoS ONE 6 (2011) e15865.
[72] C.Y. Kan, C. Petti, L. Bracken, M. Maritz, N. Xu, R. O'Brien, C. Yang, T. Liu, J. Yuan, R.B. Lock, K.L. MacKenzie, Up-regulation of survivin during immortalization of human myofibroblasts is linked to repression of tumor suppressor p16(INK4a) protein and confers resistance to oxidative stress, J. Biol. Chem. 288 (2013) 12032–12041.
[73] H.S. Kim, K. Shiraki, S.H. Park, Expression of survivin in CIN and invasive squamous cell carcinoma of uterine cervix, Anticancer Res. 22 (2000).
[74] A.A. Borbély, M. Murvai, J. Kónya, Z. Beck, L. Gergely, F. Li, G. Veress, Effects of human papillomavirus type 16 oncoproteins on survivin gene expression, J. Gen. Virol. 87 (2006) 287–294.
[75] M.H. Schoemaker, J.E. Ros, M. Homan, C. Trautwein, P. Liston, K. Poelstra, H. van Goor, P.L. Jansen, H. Moshage, Cytokine regulation of pro- and anti-apoptotic genes in rat hepatocytes: NF-κB-regulated inhibitor of apoptosis protein 2 (cIAP2) prevents apoptosis, J. Hepatol. 36 (2002) 742–750.
[76] H. Yuan, F. Fu, J. Zhuo, W. Wang, J. Nishitani, D.S. An, I.S. Chen, X. Liu X, Human papillomavirus type 16 E6 and E7 oncoproteins upregulate c-IAP2 gene expression and confer resistance to apoptosis, Oncogene 24 (2005) 5069–5078.
[77] M.A. James, J.H. Lee, A.J. Klingelhutz, Human papillomavirus type 16 E6 activates NF-kappaB, induces cIAP-2 expression, and protects against apoptosis in a PDZ binding motif-dependent manner, J. Virol. 80 (2006) 5301–5307.
[78] L. Philip, K. Shivakumar, CIAP-2 protects cardiac fibroblasts from oxidative damage: an obligate regulatory role for ERK1/2 MAPK and NF-κB, J. Mol. Cell. Cardiol. 62 (2013) 217–226.

[79] D.S. Bredt, S.H. Snyder, Nitric oxide: a physiologic messenger molecule, Annu. Rev. Biochem. 63 (1994) 175–195.
[80] S.K. Choudhari, M. Chaudhary, S. Bagde, A.R. Gadbail, V. Joshi, Nitric oxide and cancer: a review, World Surg. Oncol. 11 (2013) 118.
[81] M. De Andrea, M. Mondini, B. Azzimonti, V. Dell'oste, S. Germano, G. Gaudino, T. Musso, S. Landolfo, M. Gariglio, Alpha- and beta papillomavirus E6/E7 genes differentially modulate pro-inflammatory gene expression, Virus Res. 124 (2007) 220–225.
[82] T. Mitani, M. Terashima, H. Yoshimura, Y. Nariai, Y. Tanigawa, TGF-β1 enhances degradation of IFN-γ-induced iNOS protein via proteasomes in RAW 264.7 cells, Nitric Oxide 13 (2005) 78–87.
[83] S. Baritaki, S. Sifakis, S. Huerta-Yepez, I.K. Neonakis, G. Soufla, B. Bonavida, D.A. Spandidos, Overexpression of VEGF and TGF-beta1 mRNA in Pap smears correlates with progression of cervical intraepithelial neoplasia to cancer: implication of YY1 in cervical tumorigenesis and HPV infection, Int. J. Oncol. 31 (2007) 69–79.
[84] D.D. Thomas, X. Liu, S.P. Kantrow, J.R. Lancaster, The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O2, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 355–360.
[85] J.W. Lee, C.H. Choi, J.J. Choi, Y.A. Park, S.J. Kim, S.Y. Hwang, W.Y. Kim, T.J. Kim, J.H. Lee, B.G. Kim, D.S. Bae, Altered MicroRNA expression in cervical carcinomas, Clin. Cancer Res. 14 (2008) 2535–2542.
[86] T.H. Cheung, K.N. Man, M.Y. Yu, S.F. Yim, N.S. Siu, K.W. Lo, G. Doran, R.R. Wong, V.W. Wang, D.I. Smith, M.J. Jr Worley, R.S. Berkowitz, T.K. Chung, Y.F. Wong, Dysregulated microRNAs in the pathogenesis and progression of cervical neoplasm, Cell Cycle 11 (2012) 2876–2884.
[87] P.M. Pereira, J.P. Marques, A.R. Soares, L. Carreto, M.A. Santos, MicroRNA expression variability in human cervical tissues, PLoS ONE 5 (2010) e11780.
[88] Y. Li, F. Wang, J. Xu, F. Ye, Y. Shen, J. Zhou, W. Lu, X. Wan, D. Ma, X. Xie, Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29, J. Pathol. 224 (2011) 484–495.
[89] Z.M. Zheng, X. Wang, Regulation of cellular miRNA expression by human papillomaviruses, Biochim. Biophys. Acta 1809 (2011) 668–677.
[90] A. Pedroza-Torres, E. López-Urrutia, V. García-Castillo, N. Jacobo-Herrera, L.A. Herrera, O. Peralta-Zaragoza, C. López-Camarillo, D.C. De Leon, J. Fernández-Retana, J.F. Cerna-Cortés, C. Pérez-Plasencia, MicroRNAs in cervical cancer: evidences for a miRNA profile deregulated by HPV and its impact on radioresistance, Molecules 19 (2014) 6263–6281.
[91] I. Martinez, A.S. Gardiner, K.F. Board, F.A. Monzon, R.P. Edwards, S.A. Khan, Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma. cells, Oncogene 27 (2008) 2575–2582.
[92] W. Yuan, H. Xiaoyun, Q. Haifeng, L. Jing, H. Weixu, D. Ruofan, Y. Jinjin, S. Zongji, MicroRNA-218 enhances the radiosensitivity of human cervical cancer via promoting radiation induced apoptosis, Int. J. Med. Sci. 11 (2014) 691–696.
[93] H. Hermeking, MiR-34a and p53, Cell Cycle 8 (2009) 1308.
[94] L. He, X. He, L.P. Lim, E. de Stanchina, Z. Xuan, Y. Liang, W. Xue, L. Zender, J. Magnus, D. Ridzon, A microRNA component of the p53 tumour suppressor network, Nature 447 (2007) 1130–1134.
[95] T.C. Chang, E.A. Wentzel, O.A. Kent, K. Ramachandran, M. Mullendore, K.H. Lee, G. Feldmann, M. Yamakuchi, M. Ferlito, C.J. Lowenstein, D.E. Arking, M.A. Beer, A. Maitra, J.T. Mendell, Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis, Mol. Cell 26 (2007) 745–752.
[96] N. Raver-Shapira, E. Marciano, E. Meiri, Y. Spector, N. Rosenfeld, N. Moskovits, Z. Bentwich, M. Oren, Transcriptional activation of transcriptional activation of miR-34a contributes to p53-mediated apoptosis, Mol. Cell 26 (2007) 731–743.
[97] X. Wang, H.K. Wang, J.P. McCoy, N.S. Banerjee, J.S. Rader, T.R. Broker, C. Meyers, L.T. Chow, Z.M. Zheng, Oncogenic HPV infection interrupts the expression of tumor suppressive miR-34a through viral oncoprotein E6, RNA 15 (2009) 637–647.
[98] B. Li, Y. Hu, F. Ye, Y. Li, W. Lv, X. Xie, Reduced miR-34a expression in normal cervical tissues and cervical lesions with high-risk human papillomavirus infection, Int. J. Gynecol. Cancer 20 (2010) 597–604.
[99] X.Y. Bai, Y. Ma, R. Ding, B. Fu, S. Shi, X.M. Chen, MiR-335 and miR-34a promote renal senescence by suppressing mitochondrial antioxidative enzymes, J. Am. Soc. Nephrol. 22 (2011) 1252–1261.
[100] D.F. Mahmood, A. Abderrazak, K. El Hadri, T. Simmet, M. Rouis, The thioredoxin system as a therapeutic target in human health and disease, Antioxid. Redox Signal. 19 (2013) 1266–1303.
[101] J. Nordberg, E.S. Arnér, Reactive oxygen species, antioxidants, and the mammalian thioredoxin system, Free Radic. Biol. Med. 31 (2001) 1287–1312.
[102] P.J. Moos, K. Edes, P. Cassidy, E. Massuda, F.A. Fitzpatrick, Electrophilic prostaglandins and lipid aldehydes repress redox-sensitive transcription factors p53 and hypoxia-inducible factor by impairing the selenoprotein thioredoxin reductase, J. Biol. Chem. 278 (2003) 745–750.
[103] X.Y. Bai, Y. Ma, R. Ding, B. Fu, S. Shi, X.M. Chen, MiR-335 and miR-34a promote renal senescence by suppressing mitochondrial antioxidative enzymes, J. Am. Soc. Nephrol. 22 (2011) 1252–1261.
[104] G. Mattiasson, P.G. Sullivan, The emerging functions of UCP2 in health, disease, and therapeutics, Antioxid. Redox Signal. 8 (2006) 1–38.
[105] J. Pi, Y. Bai, K.W. Daniel, D. Liu, O. Lyght, D. Edelstein, M. Brownlee, B.E. Corkey, S. Collins, Persistent oxidative stress due to absence of uncoupling protein 2 associated with impaired pancreatic β-cell function, Endocrinology 150 (2009) 3040–3048.
[106] S. Hagiwara, A. McClelland, P. Kantharidis, MicroRNA in diabetic nephropathy: renin angiotensin, aGE/RAGE, and oxidative stress pathway, J. Diabetes Res. 2013 (2013) 173783.
[107] S. Di Castro, S. Scarpino, S. Marchitti, F. Bianchi, R. Stanzione, M. Cotugno, L. Sironi, P. Gelosa, E. Duranti, L. Ruco, M. Volpe, S. Rubattu, Differential modulation of uncoupling protein 2 in kidneys of strokeprone spontaneously hypertensive rats under high-salt/low potassium diet, Hypertension 61 (2013) 534–541.
[108] M. Truong Do, H. Gyun Kim, J. Ho Choi, H. Gwang Jeong, Metformin induces microRNA-34a to downregulate the Sirt1/Pgc-1α/Nrf2 pathway, leading to increased susceptibility of wild-type p53 cancer cells to oxidative stress and therapeutic agents, Free Radic. Biol. Med. 74C (2014) 21–34.
[109] J. Luo, A.Y. Nikolaev, S. Imai, D. Chen, F. Su, A. Shiloh, L. Guarente, W. Gu, Negative control of p53 by Sir2alpha promotes cell survival under stress, Cell 107 (2001) 137–148.
[110] H. Vaziri, S.K. Dessain, E. Ng Eaton, S.I. Imai, R.A. Frye, T.K. Pandita, L. Guarente, R.A. Weinberg, SIR2(SIRT1) functions as an NAD-dependent p53 deacetylase, Cell 107 (2001) 149–159.
[111] K.J. Herbert, A.L. Cook, E.T. Snow, SIRT1 inhibition restores apoptotic sensitivity in p53-mutated human keratinocytes, Toxicol. Appl. Pharmacol. 277 (2014) 288–297.
[112] Z. Radak, E. Koltai, A.W. Taylor, M. Higuchi, S. Kumagai, H. Ohno, S. Goto, I. Boldogh, Redox-regulating sirtuins in aging, caloric restriction, and exercise, Free Radic. Biol. Med. 58 (2013) 87–97.
[113] M. Tanno, A. Kuno, T. Yano, T. Miura, S. Hisahara, S. Ishikawa, K. Shimamoto, Y. Horio, Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure, J. Biol. Chem. 285 (2010) 8375–8382.
[114] G.J. Kops, T.B. Dansen, P.E. Polderman, I. Saarloos, K.W. Wirtz, P.J. Coffer, T.T. Huang, J.L. Bos, R.H. Medema, B.M. Burgering, Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress, Nature 419 (2002) 316–321.
[115] R.R. Alcendor, S. Gao, P. Zhai, D. Zablocki, E. Holle, X. Yu, B. Tian, T. Wagner, S.F. Vatner, J. Sadoshima, Sirt1 regulates aging and resistance to oxidative stress in the heart, Circ. Res. 100 (2007) 1512–1521.
[116] R. Puca, L. Nardinocchi, G. Starace, G. Rechavi, A. Sacchi, D. Givol, G. D'Orazi, Nox1 is involved in p53 deacetylation and suppression of its transcriptional activity and apoptosis, Free Radic. Biol. Med. 48 (2010) 1338–1346.