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Endogenous anticancer mechanisms (EACMs)

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1. ABSTRACT

Cells capable of starting the track towards cancer are probably abundant in an organism, but the likelihood of any of these cells to evolve to a deadly disease is very low. This occurs in part due to several safekeeping mechanisms shaped by evolution to detect and eliminate potential cancer-forming cells, which will be defined here as endogenous anticancer mechanisms (EACMs). Virtually any cellular process has safekeeping mechanisms that detect and correct mishaps that could evolve into potentially harmful cellular behavior, but some aspects of these mechanisms seem to have been selected by evolution to protect organisms against cancer. The mechanisms that will be discussed here and in the reviews of this series are: cell senescence, DNA repair, cell cycle control, apoptosis, autophagy, block of the invasion and metastasis cascade, block of cell reprogramming and immune surveillance. Here I will present the basic features and the importance of each EACM and review the involvement of these processes in preventing cancer growth together with their importance in cancer prevention and therapeutics.

2. ENDOGENOUS ANTICANCER MECHANISM

Endogenous anti-cancer mechanisms (EACMs) or tumor suppressor mechanisms are processes of the cell or organism that are able to decrease the incidence of cancer, or, in a more observable way, are processes which, when malfunctioning, increase the incidence of cancer (1). Under this broad definition, it is almost impossible to exclude any process of the cell or organism, although some are much more directly involved in avoiding the appearance of cancer than others.

In this issue of Frontiers in Biosciences that is introduced by the present review, eight mechanisms will be discussed for which there are strong evidence of their involvement in interfering with the development of cancers in an organism. This is not to say, however, that there are no other mechanisms that qualify as having importance in avoiding the appearance of cancer or may play specific roles in a given type of cancer or a given genetic and environmental situation.

One of the best arguments for the involvement of a given mechanism in cancer prevention is the association between alterations in a gene predominantly acting on this mechanism with altered incidence or survival in animal cancer models or, preferably, cancer patients. The difficulty with this strategy is to find genes that are exclusively involved in a single mechanism and to establish a link with survival in a broad set of cancer types. Table 1 presents a list of the EACMs that will be discussed here and exemplifies the involvement of specific mechanisms with evidence of genes that have important roles in the discussed process.

As already mentioned, probably none of the genes are totally exclusive to the process commonly associated with that gene. In some cases, like the genes associated with the early steps of DNA damage recognition, such as XPC, it is easier to link the gene to DNA repair. In other cases, such as CDKN1A (p21) or CDKN2A (Ink4A + Arf) this is much more difficult, since these genes, despite being widely linked to cell cycle regulation and senescence induction, respectively, have a pleiotropic role in the cell. Notwithstanding, in a broad sense these and other evidence, to be detailed in the individual reviews, support the involvement of the processes discussed in cancer development.

3. THE MAIN EACMs

Next I will present the main EACMs discussed in this issue and give a glimpse of the importance of the different roles of EACMs in cancer prevention, their mechanism of action and possible roles in therapeutic interventions. Obviously in the present review only a superficial view is given and a much more detailed analysis of all these points will be discussed in the individual reviews of this issue of Frontiers in Biosciences.

3.1. DNA repair

Genomic instability is a hallmark of cancer and therefore many factors involved in sensing and responding to DNA damage are altered in cancers. After the development of the concept that a single mutation in a proto-oncogene can turn it into an oncogene, the importance of DNA damage repair in cancer became straightforward. Direct evidence of the relative importance of DNA damage repair stems mainly from the association of defects in DNA damage repair mechanisms with cancer prevalence. The human hereditary diseases that are caused by mutations in genes involved in DNA repair and most clearly associated with cancer are xeroderma pigmentosum (XP), ataxia-telangiectasia (AT) and Fanconi anemia (FA).

The group of XP genes are involved mainly in nucleotide excision repair (NER) and XP patients show an increase in the incidence of skin cancer greater than 1,000-fold, with cancer development occurring usually before the age of ten (4). Data from XP as well as other NER-deficient patients indicate that this DNA repair mechanism is essential for preventing mainly UV-induced cancer. However, the fact that these patients also have an increase (12 fold) of internal tumors points to a more general role of these DNA repair mechanisms in the prevention of other kinds of tumors.

Ataxia-telangiectasia (AT) patients possess inactivation of the gene ATM, that encodes a phosphatidylinositol 3-kinase (PI 3-kinases). This protein, together with other PI 3-kinases, such as ATR or DNA-PK, are involved in activating the DNA damage response (DDR) pathways that lead to cell cycle arrest, DNA damage repair or apoptosis (3). These patients have a hypersensitivity to ionizing radiation and an increased susceptibility to T-cell leukemia and lymphomas (2), but not breast cancer (45), when compared to the general population. The increase in T-cell leukemia and lymphomas was explained by the role played by the kinases of the AT family in preserving genomic integrity during V(D)J recombination process during lymphocyte development (2), again suggesting the special importance of DNA repair mechanisms and DNA damage responses to prevent cancer under genomic stress conditions.

Fanconi anemia (FA) is a rare multigenic disorder caused by alteration in the FANC gene family composed of 14 genes, involved, directly or indirectly, in the appropriate assembly of DNA repair nuclear foci. FA patients possess a 1,000 fold increase in the incidence of leukemia when compared to the general population (46). BRCA2 is a FANCD1 complementing gene (47) and defects in BRCA1 or 2 are associated with an increase in breast cancer incidence (48). In mice, BRCA1 plus p53 deletion leads to the development of breast cancer (49).

Modulation of DNA damage repair processes bears a great therapeutic potential in cancer. Inhibition of MGMT (O6-methylguanine-DNA methyl-transferase), involved in the removal of alkyl adducts from the O6-position of guanine, has improved the efficacy of alkylating agents such as BCNU, CCNU and temozolomide in several cancer types (50). Inhibition of base excision repair by binding to DNA abasic sites or inhibition of key enzymes of this process was also shown to enhance the cytotoxicity of several drugs in aggressive cancers such as fibrosarcomas and glioblastomas (51). Therefore, increased DNA repair may be the origin of several cases of resistance of cancer cells to DNA damage inducing treatments and gaining the capacity to modulate DNA repair can be a further step to increase the efficacy of cancer therapy.

3.2. Cell cycle checkpoints

The vast majority of cells in an adult mammal are not actively cycling (52). Therefore, deregulation of the cell cycle was one of the first processes to take the blame for cancer development. Although uncontrolled cell cycle is a sine qua non of cancer, the existence of other EACMs described here suggest that in order for a cancer to develop, much more has to go wrong in addition to a deregulated cell cycle.

The cell cycle is controlled by a set of genes that involve cyclins, CKIs (CDK inhibitors), kinases, most prominently the CDKs (cyclin-dependent kinases), phosphatases, and transcription factors. Alterations in

Table 1. EACM and genes that support their anticancer role

EACM	Genetic or patho-physiologic evidence.	Evidence from Patients	Evidence from animal models
DNA Repair	Ataxia-telangiectasia (AT) – activation of the DNA damage response pathway	Patients with defects in ATM gene have an increase in T-cell leukemia and lymphoma (2) incidence compared to normal population	ATM KO mice show a predisposition to develop lymphoid malignancies (3).
	Xeroderma pigmentosum (XP) gene family - several functions in nucleotide excision repair (4)	Defects in XPD are associated with drastic increase in melanoma (4)	Xpa, Xpc, Xpe KO mice show increased skin cancer induced with UV light or chemical carcinogens (4)
Cell Cycle Checkpoints	Rb - Binds to E2F1 – Rb is phosphorylated by CDKs leading to E2F1 target gene activation	Inactivation of Rb is associated with the development of retinoblastoma and other cancer types (5).	Conditional KO of Rb plus p53 increases the speed of osteosarcoma development (6).
	Cdkn1A - $p21^{WAF1/Cip1}$ - Inhibitor of CDK2, 4 and 6, but also exerts several CDK-independent functions (7)	Both positive and negative correlation between the expression of p21 and survival of patients with diverse types of cancers (7).	Deletion of Cdkn1a induces late development of tumors of various origins, but mice are less susceptible to radiation-induced carcinogenesis. Deletion also accelerates the formation of several cancers when other tumor suppressor genes, such as p53, Ink4 or Rb, are co-deleted (7).
Senescence	Telomerase (Tert) - Telomere synthesis	Gain at chromosomal region containing Tert is the most frequent genetic event in lung cancer (8) and the presence of a silent mutation in the Tert gene is associated with basal cell carcinoma, lung, bladder and prostate cancer (9).	Overexpression of Tert induces several kinds of tumors (10, 11) and mice KO for Tert have a reduced incidence of several types of cancer (10, 12).
	Cdkn2A (INK4A and Arf) - CDK inhibitor (Ink4A) and p53 activator through downregulation of MDM2 (Arf)	High incidence of mutation in the Cdkn2A locus in glioblastomas, lung and pancreatic adenocarcinomas, melanomas and several other types of cancer (13).	Deletion of Arf or Ink4a increases basal cancer as well as the development of cancers induced by several oncogenes (14).
Apoptosis	Bcl-2 - Blocks cytochrome release from mitochondria	Genomic translocation that increases Bcl-2 expression associated with lymphomas (15)	Mice bearing a minigene that recapitulates the Bcl-2 translocation found in patients develop lymphomas. Leukemia develop when combined with Myc expression (15).
	XIAP - Block of caspases and ARTs -antagonists of IAPs including XIAP	Higher expression of XIAP found in several types of cancer and an indication of worse prognosis (16, 17). ARTs is lost in the majority of acute lymphoblastic leukemias (18) and expression inversely correlates with survival of patients with astrocytic tumors (19).	While no report was found on altered sensitivity of XIAP KO to cancer, KO of ARTs was shown to increase cancer development in a Eμ-Myc genetic background (20).
Autophagy	Beclin1 (Atg6) –induces autophagosome nucleation through formation of an initial complex with PI3K III	Beclin1 is monoallelically deleted in breast, ovarian and prostate cancer (21). Beclin 1 is positively correlated with survival of patients with non-Hodgkin lymphomas, pancreatic ductal adeno-carcinoma and esophageal squamous cell carcinoma (22-24). In nasopharyngeal carcinoma, higher expression of Beclin-1 correlated with poorer overall survival (25).	Beclin1 monoallelic deletion induces lung and liver cancer as well as lymphomas in mice (26, 27). enforced expression of beclin 1 and atg5 inhibits the formation of human breast tumours in mouse models
	Atg4C – cleaves Atg8/LC3, a fundamental step to autophagosome formation	No evidence found in patients for ATG4C	Atg4C KO increased susceptibility to develop fibrosarcomas induced by chemical carcinogens (28).
Block of Cell Reprogramming [a]	Myc – pleiotropic transcription factor involved in cell reprogramming and several other biological functions.	Is overexpressed in several types of cancers and expression inversely correlates with survival of several of these cancers (29).	Eμ-Myc mice, in which the Myc gene is regulated by an Ig promoter, develop several types of cancers such as lymphomas and leukemias (30).
	p53 – pleiotropic transcription factor involved in most of the EACM. The description of its involvement in blocking cell reprogramming supports the existence of a EACM that acts on differentiation (31).	High incidence of deleted or mutated p53 in a variety of human cancers(32). Association between p53 mutations and embryonic stem cell signatures in breast and lung cancers (33).	Mice having an extra copy of p53 are more resistant to cancer development and p53 KO increases incidence of several types of cancer (34). As stated, the relative role of differentiation in the effects of this pleiotropic gene is very difficult to assess.
Block of Invasion and Metastasis Cascade	E-cadherin – transmembrane glycoprotein involved in epithelial intercellular adhesion	Decreased expression of E-cadherin is associated with poor prognosis in several types of cancer (35, 36).	Loss of E-cadherin increases metastasis in a mouse models of breast carcinoma (37).
	"Metastamirs" (large set of miRNAs that act on several aspects of the invasion and metastasis cascade.	Several miRNAs that act on the invasion and metastasis cascade are differentially expressed in primary and metastatic tumor, including miR21, miR31(38-40)	Anti-miR21 or miR31 expression block metastasis in several cancer models (38, 39).
Immune Surveillance	Immunodeficiency virus (HIV or SIV)	HIV patients have up to 100 times higher incidence of Karposi´s Sarcoma and Non-Hodgkin´s Lymphoma (41).	Monkeys infected with simian immunodeficiency virus develop lymphomas (42).
	Immuno-suppression due to transplant related therapies.	Immunosuppressed renal transplant patients have higher incidence of several cancer types, specially non-melanoma skin cancer (43).	Immunosupressed mice have a higher incidence of non-induced as well as carcinogen-induced tumors (44).

[a]No gene exclusively involved in cell reprogramming was found. Therefore two very important genes involved in reprogramming and which have a strong association in cancer were used as examples. It has to be kept clear that these genes are by no way specific to cell reprogramming.

several of these genes have been linked to cancer, among which the deletion or inactivating mutations of CKIs, such as p21Cip/Waf (CDKN1A) and p16INK4A (CDKN2A) are the best documented (53). Deletion of the retinoblastoma (Rb) gene, which encodes the best characterized CDK substrate, is linked to cancer development in patients, but the link in animal models is less clear (5, 6).

p21Cip/Waf is an example of the difficulty to define the role of a given process in cancer development. While the role as an inhibitor of CDK2/4 and 6 is firmly established and explains several observations concerning the link between the deletion of p21 and the development of cancer, other functions, such as the inhibition of apoptosis, can turn this gene into an oncogene, rather than its more traditional role as tumor suppressor gene (7).

Cyclin D1 is the most studied cyclin in cancer. This cyclin is overexpressed either by chromosomal translocation leading to transcriptional activation or by amplification of the CCND1 locus in several types of cancer, including lymphomas, breast, esophageal, bladder, lung and squamous cell carcinomas (54). Other cyclins, such as D2, D3, E, and A were also found associated with cancer development and higher expression of these cyclins correlates with worse prognosis (54).

Cell cycle arrest is a long standing objective of cancer therapeutics and several anticancer drugs currently in use work, directly or indirectly, by inhibiting the cell cycle arrest. The more direct inhibitors of the cell cycle in therapeutic use are the drugs that stabilize or destabilize microtubules, such as taxol and vincristine, therefore blocking the cytoskeleton dynamics required for cell division (55). However, despite the development of several specific inhibitors of CDKs, no breakthrough therapy based on CDK inhibition has made it through to the clinic yet. Notwithstanding, combinations of CDK inhibitors with several drugs in clinical use have produced promising results in clinical trials (56, 57). Unfortunately, high binding of the drugs to serum proteins and the need of chronic dosing schemes still hamper their advance towards clinical approval.

3.3. Senescence

Cellular senescence is a state of stable, and in most cases irreversible, cell cycle arrest that is prevalent in pre-malignant tumors, but largely absent in malignant tumors (58, 59). Three main cellular events can induce senescence: 1. large number of previous cell divisions, at least *in vitro*; 2. DNA damage and 3. oncogenic signals. The first is the well-known process of replicative senescence (RS), which is induced by telomere erosion that occurs with each cell division, and can be prevented by the ectopic expression of the catalytic subunit of the telomerase holoenzyme (hTert), responsible for the elongation of telomeres (60-62). The second and third events, collectively called premature senescence, occur prior to the stage in which telomeres are eroded and can be induced by oncogenes, such as HRasG12V or Myc, being often referred to as oncogene-induced senescence (OIS). Premature senescence can also be induced by DNA damage from radiation or drugs such as doxorubicin or resveratrol (60, 61, 63, 64). The premature senescence induced by oncogenes requires the activation of the Cdkn2A locus (p16Ink4A and p14ARF) and p53 either through direct signaling from oncogenes (65, 66) or through DNA damage and activation of the DNA damage response (67, 68).

The main data supporting the importance of senescence in cancer development comes from studies in genetic models of cancer and aging. There is a vast body of evidence linking the level of telomerase activity with cancer development – Tert over-expressing mice are more prone to several types of cancer (10, 11), whereas deletion of Tert turns the mice more resistant to cancer induction (10, 12). Inactivation of the Cdkn2A locus blocks senescence induction by oncogenes such as BRAFV600E or HRASG12V, leading to the progression to malignant tumors (58). Deletion of this locus also increases basal cancer occurrence as well as development of cancers induced by several oncogenes (14).

In patients the main data regarding the importance of senescence in cancer comes from the presence of senescence in pre-malignant tumors from lung, pancreas and melanocytic nevi when compared to the absence of senescence in corresponding malignant stages (13). Additional support for the role of senescence comes from genetic alterations in loci involved in senescence, such as amplification at the chromosomal region containing Tert which is the most frequent genetic event in lung cancer (8) and the high incidence of deletion or mutation in the Cdkn2a locus in glioblastomas, lung and pancreatic adenocarcinomas and several other types of cancer (13).

A surprising development in the field of senescence was the discovery of soluble factors able to reinforce senescence in a paracrine manner. The senescence-messaging secretome (SMS) or senescence-associated secretory phenotype (SASP) is composed of insulin growth factors (IGFs) and their binding proteins (IGFBPs), interleukins (ILs), interferon γ (IFN γ) and transforming growth factor-β (TGFβ) (69). This phenotype can be induced by over-expression of an oncogene, such as BRaf in melanomas, where senescence is induced by secretion of IGFPBP7 (70).

There is good evidence that several DNA damage-inducing drugs work, at least in part, through induction of senescence(65). Development of drugs focused on the induction of senescence rather than induction of cell cycle arrest or apoptosis may lead to better therapeutic options.

3.4. Apoptosis

From early on, apoptosis was recognized as a very refined process for eliminating unwanted cells during development and therefore rapidly established itself as a very strong candidate as an anticancer mechanism. The discovery that apoptosis could be triggered in an adult organism by internal hazard signals, such as DNA damage or unbalanced oxidative stress as well as external signals sent mainly by the immune system, such as TNF or Fas

ligand, led to the general acceptance of apoptosis as the major EACM (15).

The confirmation of apoptosis as an EACM was established with the discovery of genes involved in apoptosis whose alteration correlated with cancer incidence and aggressiveness. A key discovery was Bcl-2, which broadened the range of action of oncogenes from growth promoting to include resistance to cell death. Bcl2 gene is over-expressed in lymphomas and has a negative role in the liberation of cytochrome C from the mitochondria therefore protecting cancer cells from undergoing apoptosis. The discovery of other genes that work predominantly on apoptosis and whose expression correlates with survival, such as XIAP, Survivin and TRAIL, confirmed the importance of apoptosis in preventing the appearance of cancer. Additional support came from transcriptional regulation of genes involved in apoptosis induction, such as Noxa and Puma by p53 (71), despite the intrinsic difficulty in establishing the relative role of these targets among the large set of p53 regulated genes. An indication of the difficulty in establishing a bona fide EACM are the recent observations that some apoptosis inducers, such as Fas or PUMA, can, under some circumstances, be tumorigenic (72).

Another group of genes involved in the fine regulation of caspases, the major effectors of apoptosis, are the IAPs (inhibitor of apoptosis). Over-expression of IAPs is linked to development and resistance of several types of cancer to anticancer drugs (73) as is the case of XIAP, whose high expression is an indication of worst prognosis in leukemia and renal carcinoma (16, 17). IAPs can be endogenously inhibited by antagonists, such as Smac/Diablo, Omi/HtrA2 and Sept4/ARTS. One of these inhibitors, ARTs, is less expressed in acute lymphoblastic leukemias (18) and expression of this protein inversely correlates with astrocytic tumor aggressiveness and patient survival (19). Mice in which this gene was deleted have accelerated tumor development in a Em-Myc background accompanied by an increased pool of cancer stem cells (20).

Several of the most efficient cancer therapies, such as radiotherapy and DNA damaging agents, act by inducing cell death, which includes death by apoptosis. However, few of these therapies were designed to selectively activate the apoptosis machinery. Furthermore, much of the resistance to current therapies may come from general resistance to apoptosis, which positions the development of drugs specifically modulating the apoptotic process as one of the main bids to tame this resistance in order to increase therapeutic efficacy.

The therapeutic strategies focus on direct activation of apoptosis with TNF, TRAIL or CD95 (74). Another therapeutic development directly linked to activate (or block the inactivation of) apoptosis are drugs able to inhibit the Bcl2 anti-apoptotic family (75) or IAPs based on the binding of the endogenous inhibitor SMAC (called SMAC mimetics) (16, 17, 74).

3.5. Autophagy

Autophagy, more specifically, macroautophagy, is a process of degradation of intracellular compartments in which organelles and cytoplasm are encapsulated into a double membrane which later is fused to the lysosome for degradation. Autophagy can act on different stages of cancer and may have opposing roles in cancer development, survival in hypoxic conditions and treatment.

At first, autophagy was hailed as promoting the survival of solid cancer cells, mainly in oxygen and nutrient starved regions of a solid tumor where the autophagic process provides energy for the survival of these cells until energy sources are restored (76). More importantly, high levels of autophagy are induced by radio or chemotherapy and under these circumstances autophagy normally has protective roles (77) although there are cases in which induction of autophagy seem to be part of the mechanism of treatment induced death (78).

On the other hand, deletion of genes important for autophagy such as beclin 1, bif, Atg4 or UVRAG generate a tumor-prone phenotype in mice, suggesting an anticancer function of autophagy (79). Furthermore, from the five studies found which correlated Beclin1 expression level with patients survival, three reporter a positive correlation and one reported a negative correlation (22-25). A study in melanomas reported an interesting biphasic survival pattern, probably reflecting the dual role of autophagy in cancer (80). Together, these data from patients and mice point to an important role of the autophagy affected by Beclin 1 reduction in cancer prevention.

A second set of data supporting the involvement of autophagy in cancer comes from the crosstalk with other important EACMs. Senescence induced by oncogenic Ras leads to activation of hallmarks of autophagy and if this process is blocked, senescence is somewhat retarded, suggesting that these mechanisms are intertwined at the level of late execution (81). Autophagy interplay with apoptosis is complex and context-dependent. Induction of autophagy may block apoptosis as well as work as a failsafe mechanism in apoptosis-deficient cells (78). Although promising, these interactions are presently not clear enough to be therapeutically explored.

Autophagy is induced by several anticancer agents, but it is far from clear if this is a response to the stress induced by these agents, and therefore part of the mechanism of protecting the cancer cell, or if autophagy is fundamental for the mechanism of cell elimination (82). Some studies point to a beneficial therapeutic effect of inhibiting autophagy in a diverse group of tumor types and treatments (79) suggesting that autophagy protects cancer cells under these circumstances. In other cases, blocking of autophagy suppresses cancer cell death (78), positioning autophagy as part of the mechanism of cell death. Therefore, pharmacological modulation of autophagy may well need to wait for personalized therapy in which the genetic makeup of the tumor and its sensitivity to drugs are evaluated prior to the actual therapy.

3.6. Block of Cell Reprogramming

It is now well accepted that several, if not all, cancer types possess a sub-population of cancer stem cells (CSCs) which are, in most cases, fundamental for the growth and maintenance of the cancer (37). It is still an open debate, however, whether these CSCs originate from normal stem cells or from differentiated cells. The discovery that four specific transcription factors, Myc, Oct4, Sox2 and Klf4, are able to de-differentiate cells (83) supports the possibility for the latter hypothesis (84).

Considering the increased risk of cancer posed by de-differentiated cells, it came as no surprise that several genes linked to cancer, such as p53, p21, p14ARF, p16INK4A, were found to be able to block the de-differentiation of cells (31). Additionally, breast cancers with inactivated p53 have a stem cell transcriptional signature in breast and lung cancer (33). This is not a proof, however, for the involvement of de-differentiation in cancer prevention, since the above genes are involved in other fundamental EACMs, such as cell cycle control and senescence. Involvement of these pleiotropic genes in cancer are not an evidence in favor of one or the other process, but the link of these fundamental tumor suppressor genes with the block of de-differentiation is an indication that de-differentiation is to be avoided under certa*in situ*ations, which may include instances in which cancer development is a possible outcome (31).

The concept of cancer as a tissue relying on a pool of stem cells for its renewal led to the observation that these cells are normally more resistant to radio (85) or chemotherapy (86) and therefore may be responsible for more aggressive recurrences. The CSC concept also led to the proposal of differentiation therapy, designed to specifically differentiate CSCs (87). This is potentially a much less aggressive and probably more effective therapy than killing all cancer cells.

3.7. Block of invasion and metastasis cascade

In order to form metastases, cancer cells have to surpass several physical and biological barriers, which include invasion through the host tissue to reach and enter into the lumina of blood vessels, survival in the absence of adhesion, invasion of the ectopic organ and active growth in the new microenvironment of this organ. Any of these barriers is unsurpassable to a normal cell, but a series of biological events – collectively named the "invasion-metastasis cascade" select cancer cells capable of this feat (88). One important step for the development of the invasive phenotype by tumor cells is the epithelial-mesenchymal transition (EMT), in which the intercellular adherent junctions are dissolved through, among other mechanisms, downregulation of E-cadherin expression (89).

Anoikis, the cell death due to lack of adhesion, is a mechanism that probably evolved to safeguard the organism against the movement of cells from solid tissues through the organism in the bloodstream (90) but the exact contribution of anoikis in avoiding cancer is difficult to assess due to the overlap of this mechanism with apoptosis.

The discovery of an miRNA that inhibits breast cancer metastasis, acting on several steps of the invasion and metastasis cascade, is one of the first supports of an anticancer mechanism that seems to have evolved to quell metastasis (39, 40). This may be the beginning of the discovery of other such genes and the development of therapies aimed at blocking metastasis, which is, by far, the deadliest aspect of cancer (38-40).

The presence of endogenous inhibitors of angiogenesis may also be part of the arsenal of the organism to impede the growth of ectopic cells in a given tissue. When these endogenous inhibitors were deleted in mice, no clear physiological defect was detected, but a restriction of the growth of implanted tumor was observed. The opposite was observed when these genes were over-expressed (91).

The existence of defined microenvironments in different tissues very probably is a requirement for the function of these tissues. However, this also produces a restriction of the growth of ectopic cells, but which is overcome by the cancer cells either by turning itself independent of certain signals or by secreting the factors that mimic their microenvironment of origin (92). However, not enough evidence are available to defend the concept that these specific microenvironments were evolutionary shaped by the selective pressure of cancer.

3.8. Immune surveillance

Detection of non-self is one of the central hallmarks of the immune system. Therefore, cancer poses a highly challenging problem because cancer cells are "self" with minor modifications. The immune system can detect the so called tumor-specific antigens (TSAs) and eliminate the cells presenting these antigens in a process called immune surveillance. The immune system can also protect the organism against cancers in a more indirect way by eliminating or suppressing tumor-inducing viruses or by the elimination of pathogens and prompt resolution of inflammation that can prevent the establishment of an inflammatory, pro-tumoral, environment (93).

The importance of this immune surveillance was recognized with the immunosuppressive states observed in advanced HIV (41) and organ transplant patients (43). In both instances, cancer development was several times higher in these patients when compared to matched controls, suggesting that immune surveillance is constantly active in eliminating potential cancerous cells.

The observation that tumors from immunocompromised animals are much more immunogenic than tumors from immunocompetent animals led to the proposal of the immunoediting hypothesis, which proposed that the selec-tive pressure that the immune system imposes on the tumor cells significantly alters the tumors, eventually leading to the escape from the immune system, for example due to low immunogenicity of the tumor cell or depletion of the hosts immune system (93).

Understanding of the interplay between the tumor cells and the immune system led to the development of several therapeutic strategies aimed at increasing tumor

immunogenicity or reactivating the immune system against the cancer cells. Unfortunately, this has not been as easy as vaccination against viruses, and complex interventions, probably personalized, are required to effectively reactivate the immune system against cancer cells (94).

3.9. Other mechanisms that may be involved in preventing cancer

This issue of Frontier in Bioscience aims to explore important anticancer mechanisms, but, as is so common in science, it is impossible to cover all aspects of a topic. There certainly are several other mechanisms able to reduce cancer, either by producing an overall beneficial effect on the cell or organism, or by specifically targeting a cell process that can, if modified, contribute to tumorigenesis.

Just to cite two examples, control of the redox state is important to keep mutations low as well as to maintain a functional cellular environment. Additionally, metabolism is considerably altered in cancer cells, and it is likely that specific mechanisms impede metabolic alterations that could lead to tumorigenesis.

Therefore, although 8 mechanisms are covered by reviews in this issue, this is not to say that other mechanisms, even those not conceivable today, are not important for quelling tumorigenesis and may be part of our understanding and perhaps therapeutic options in the future.

4. EVOLUTION OF EACMs

Cancers are often thought to be selectively neutral since most of the individuals they kill are post-reproductive (95). But this statement does not take into account that this late onset only happens due to the presence of EACMs that block cancer appearance and development. Malfunctioning of EACMs, which is observed in several genetic disorders, lead to cancer related death in pre-reproductive organisms and therefore selective pressure from cancer may have played a role in fine-tuning the EACMs discussed here.

Cancer has been recorded in several organisms such as mollusks, arthropods, fishes, amphibians, reptiles and mammals (95). This antiquity of appearance should have provided enough time for the selection of EACMs. One of the few studies on co-evolution of pro- and anticancer genes was done in two closely related species of fish, in which one species has pro- and anticancer loci whereas the other lacks both. Hybrids in which only the pro- cancer locus is present show high susceptibility to melanomas, suggesting that a tumor suppressor locus was selected to counteract the appearance of the oncogenic locus over a relatively short time, given the similarities between these two species (95-97).

Most cellular processes are tightly regulated, which is central for impeding the appearance and progression of cancer. Take the control of the cell cycle for example - it produces the right amount of a given cell type in a given moment, according to the requirements at a specific location. This alone calls for the integration of a multitude of signaling pathways initiated by external as well as internal signals that impinge on the cell cycle control machinery. Some of these signaling pathways have evolved to detect abnormal levels of mitotic signaling, activating an irreversible cell cycle block i.e. senescence (98). This mechanism seems to have been shaped by selective pressure to tame cells gone wild, therefore preventing their uncontrolled growth and cancer formation. Therefore, the main selective pressure for the presence of cell senescence seems to have been to combat cancer formation and progression, while other selective pressures probably played a small role in the evolution of this process (Figure 1A).

Maintenance of DNA integrity is certainly a matter of life and death for the organisms, and in a broader sense, for the species. However, the relation between DNA damage and/or defects in DNA repair and cancer suggest that this mechanism also plays a fundamental role in cancer prevention, especially in tissues submitted to high genomic stress such as skin cells exposed to sunlight and cells which possess high rates of recombination during development, such as lymphocytes (2). Notwithstanding, genomic stability is such a fundamental feature of life that selective pressure other than cancer probably were more important for the evolution of DNA repair.

Other processes are very important for preventing cancer, but also have prominent functions in several fundamental biological processes. Apoptosis, for example, plays an elementary role in shaping several organs during development by eliminating surplus cells (99). Ever since apoptosis was discovered, its role as an anticancer mechanism was one of the justifications for the maintenance of a self-killing process in evolution, but the task of precisely accessing the relative importance of the anticancer mechanism in shaping apoptosis during evolution is difficult due to the lethal phenotypes of deletion mutants of the central core of the apoptotic machinery, i.e. caspases 3, 8, 9 and Apaf-1 (15, 100). This embryonic lethal phenotype of dysfunctional apoptosis suggests that development rather than cancer was the main selective pressure that shaped the core of the apoptotic process (Figure 1B). On the other hand, the mild phenotypes of Bcl family knockouts (100) suggest that the fine tuning of its basic mechanism was not shaped by development, probably being shaped by other selective pressures, such as cancer. Of note, from the genes presented in Figure 1B, only deletion of Bad and overexpression of Bcl2 have phenotypes under basal growth conditions that include cancer, suggesting that among these “accessory” apoptotic genes, only a few are directly involved in avoiding cancer. Other processes, such as the cell cycle regulation, also seem to have a central core, probably composed of a single CDK, one of two cyclins and CKIs (101), but is actually performed by 11 isoforms of CDKs, 31 ciclins and 7 CKIs, also suggesting that a central core evolved for the basics and several gene duplications were maintained during evolution to provide the necessary fine tuning important for other processes, among which is cancer prevention.

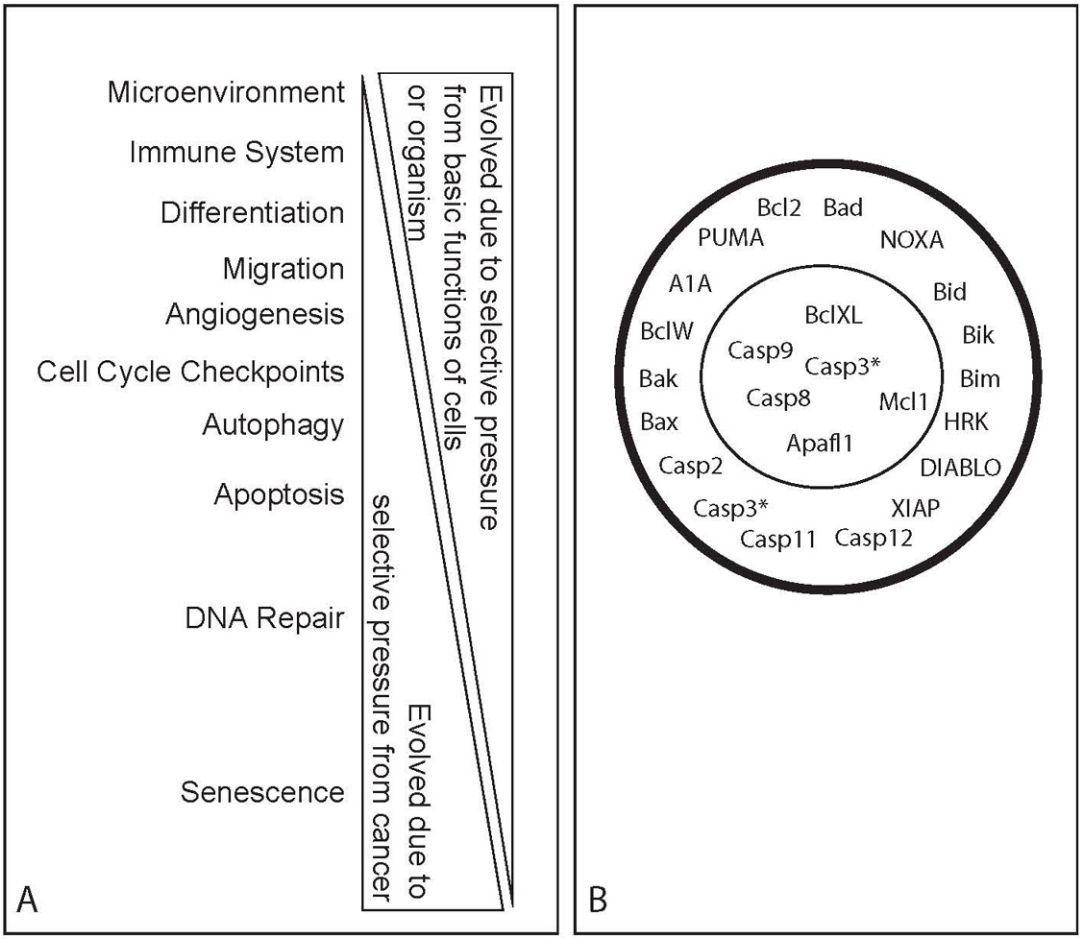

Figure 1. A. Putative importance of avoiding cancer as a selective pressure in evolution for the appearance of specific anticancer mechanism. The purpose of this figure is to point out that selective pressure from cancer had different weights in shaping these mechanisms, but the order and position of the different mechanisms are hypothetical. B. Phenotypes of gene deletion of caspases (107) and Bcl family member (108). At the center are embryonic lethal phenotypes while at the outer circle are knockouts with several phenotype or absence of clear phenotypes. *Caspase 3 KO is lethal depending on the genetic background (107).

In Figure 1A, the issue that selection to prevent or postpone death due to cancer had different weights in the evolutionary shaping of different mechanisms is raised. However, the order and location of each mechanism is hypothetical as there is no direct experimental evidence for the relative importance of cancer rather than other basic processes of the cell or organism in selecting these mechanisms.

On the upper side of the chart of Figure 1A, we can ask how much of the function of the immune system was shaped by evolution due to a cancer-related selective pressure. Certainly, the "other" functions of the immune system, i.e. eliminating viruses, bacteria and fungi, were much more important for the survival of an individual than the elimination of cancer cells. But, the capability of the immune system to recognize and eliminate transformed cells, the so called immune surveillance (93), suggests that this function was somehow shaped by a selective pressure of cancer and therefore this aspect of the immune system can be regarded as an EACM.

On the other hand, basic cellular mechanisms such as differentiation, migration, angiogenesis, autophagy and the basic control of the cell cycle probably were shaped primarily by development or other important aspects of the biology of the cell or organism rather than cancer, although, here again, fine tuning of some aspects of these processes might be influenced by selection due to cancer.

One interesting aspect of EACMs is the concentration of several tumor suppressor genes on short stretches of the genome. This is the case of the genes cdkn2a, cdkn2b and mir-31 in the 9p21.3 locus. Even more striking is the production of two important tumor suppressors proteins (p14ARF and p16INK4A) from a single mRNA, (cdkn2a) through alternative reading frame usage (102). This begs for the question of why evolution has not selected organisms in which these genes were in different loci, and therefore were not amenable to concurrent deletion, as so frequently happens in cancers in this locus. Additionally, the observation that duplication of p53 has cancer protective effects (103) also suggests that if

cancer were a dominant evolutionary driving force, duplication events of parts of the genome containing p53 would have been stabilized in the population.

Although some of the anticancer functions presented by the EACMs may be a by-product of mechanisms that were selected for by evolution, several aspects described in this issue seem to be too cancer specific to be regarded as simple by-products. Therefore I present the concept that, although the central aspects of the EACMs may not have been shaped by evolution due to cancer selective pressures, several aspects of the fine tunings of these mechanisms may have been shaped by the selective pressure of cancer.

5. CROSSTALK AMONG EACMs

Among these anticancer mechanisms, there are those that lead to a final fate of the pre-cancerous cell, as is the case of apoptosis. The majority of the EACMs, however, interact among themselves in order to reach the desired endpoint of eliminating the pre-cancerous or cancerous cell. This is clearly the case of DNA damage, which activates cell cycle arrest, senescence, apoptosis and/or autophagy, leading to the full recovery or elimination of the damaged cell.

It is also important to discuss that several of these mechanisms overlap in their induction and execution mechanisms, and that sometimes a single cell possesses markers of more than one mechanism. This is the case, for example, of apoptosis and autophagic cell death, whose features can be observed in single cells. Establishing exactly if one leads to the other and therefore co-exist for a while or if they simply co-occur is generally very difficult but very important for the understanding of tumor biology and therapy. Despite these grey zones and overlaps (104), in the series of reviews introduced here we normally consider these mechanisms as a fairly well defined process. However, as so many other complex biological processes, an attempt has to be made to view individual mechanisms as composing a whole, and integrated process. Therefore this issue will end with an article focusing on the integration among the EACMs described here, authored by the main authors of the individual reviews.

6. MODULATION OF EACMs IN CANCER THERAPY AND PROPHYLAXIS

(Re)activating or inhibiting a given EACM in therapeutic intervention is a complex matter. Some EACMs, such as DNA repair, also prevent the action of several, mainly DNA damaging based, therapies. Therefore, their inhibition is a strategy that normally increases the effectiveness of these kinds of drugs. On the other hand, therapeutic intervention often acts through cell cycle arrest, apoptosis or senescence, and activation of these processes is normally wished for. Differentiation therapy has been suggested and tested in models organisms (87, 105), and in humans the use of retinoic acid can be considered a differentiation therapy with considerable success, mainly when combined with other drugs.

However, in most cases the answer to the question of whether to activate or inhibit a given EACM is not as clear-cut as we would hope for. Even an EACM, such as the cell cycle control, that we would think to be obvious that its activation is preferred in therapeutic intervention, may not be that clear. Several drugs work better if cancer cells do not arrest in the cell cycle, progress and eventually die of mitotic catastrophe (106).

The main advantage of the establishment of the concept of EACMs acting during the whole life history of an organism may be their chronic modulation with the objective of increasing the surveillance towards potential cancer-forming cells and their elimination. This could produce therapies aimed at increasing the efficacy of the EACMs and therefore have a prophylactic effect in avoiding cancer.

It is reasonable to suppose that a better understanding of the workings of the individual EACMs and their interactions during the carcinogenic process will lead to clinically relevant information and even pharmacological therapies aimed at modulating EACMs so as to increase the endogenous defense of the organisms against cancer in a prophylactic fashion.

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Abbreviations: Arf: alternative reading frame, ARTS - Apoptosis-Related Protein in TGF Beta-Signalling Pathways, ATM: ataxia-telangiectasia mutated, Bcl: B-cell lymphoma, Bif - Bax-interacting factor-1, BRCA1: breast cancer 1, CDK – cyclin-dependent kinase, CKI – CDK inhibitor, EACM: Endogenous anticancer mechanism, EMT - epithelial-mesenchymal transition, IAP – Inhibitors of Apoptosis, INK4 – Inhibitors of CDK4, Klf4 - Krueppel-like factor 4, KO – knockout, miRNA: microRNA, Oct4 – Octamer 4, PI3k – phosphoinositide 3-kinase, Rb: retinoblastoma, SMAC – second mitochondria-derived activator of caspase, Tert: Telomerase, TNF – Tumor Necrosis Factor, TRAIL – TNF-related apoptosis-inducing ligand, TSAs - tumor-specific antigens, UVRAG - UV radiation resistance-associated gene protein, XIAP: x-linked inhibitor of apoptosis protein, XP: xeroderma pigmentosum

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