3 Personalized Prevention Strategies to Defeat Cancer

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3.1 Introduction

Personalized treatment is, surely, one of the most urgent needs in the clinical strategies of prevention and cure of tumors.

New possibilities have been opened by the latest results [1] of the research on the aging changes specific for gender in the regulation of the redox-immune system homeostasis.

It has been demonstrated that Trx1/CD30 redox immune system (Trx1/sCD30) is a double target biomarker; it is both aging-related and specific for gender and can be used to establish the very early risk for cancer development or its progression.

Trx1/soluble CD30 (Trx1/sCD30) has been proposed as a new double pharmacological target for treatment to restore the redox-immune system homeostasis during aging and the normal levels of Trx1, RTrx1, sCD30, and cytokines T regulatory (Treg), T helper1, (Th1), Th9, and Th17. These are functional biomarkers of extracellular and intracellular pathways of Trx1/sCD30. Furthermore, the polymorphisms of killer immunoglobulin-like receptors (KIRs) and receptors for the Fc domain of IgG (FcγR) FcγRIIa-131H/R and FcγRIIIa-158V/F have been proposed as clinical stratification parameters to personalize the prognostic biomarkers in non/low/high disease risk indices.

3.2 The Thioredoxin1 System

The redox control of the cell physiology is one of the most important regulatory mechanisms in all the living organisms. The Trx1/RTrx1 system is a relevant regulator of the redox-mediated cell reactions of the whole organism.

Mammal cells contain two Trx systems. The first being Trx1/RTrx1 is normally localized in cytoplasm, but in stress conditions, it could migrate in the nucleus (inducing the transcription and transduction of target genes) or it could be secrete in the extracellular environment [2] and take part, in this way, to the network of the immune system. The second one, Trx2/RTrx2, localized in mitochondria and in the endoplasmathic reticulus, regulates the cell apoptosis [3]. In addition, literature reported other Trx systems: the Testis/sperm-specific, localized on the spermatids (Sptrx-1, Sptrx-2, and Sptrx-3), and the Trxl-2, located in the lungs and in other ciliate tissues [4].

Trx1 is a thermostable protein (constituted of 108 amino acids) that is largely distributed in all the living organism, from bacteria to mammals. It contains an S-S bridge, it does not contain metal, and it has a catalytic domain that is a donor of hydrogen for redox reactions [5, 6] (Fig. 3.1). The Trx1-reduced form is able to reduce protein disulfides by using their two active cysteine site.

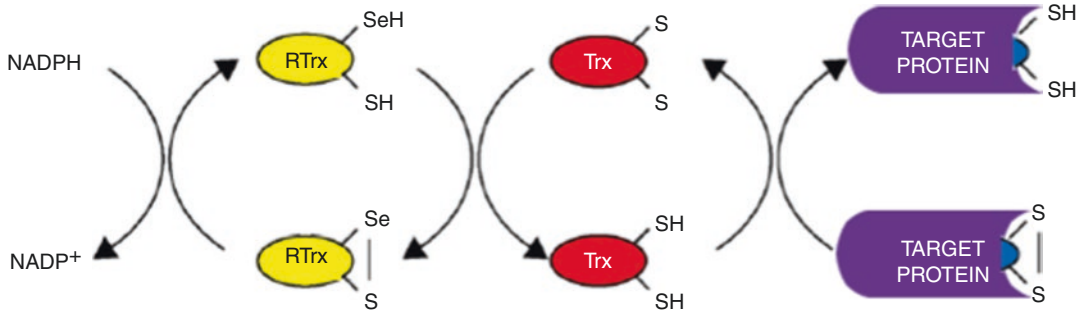

Fig. 3.1 Thioredoxin 1 (Trx1) system. Trx1 reduces protein disulfides using their two active site cysteines, and upon reduction of target proteins, it is itself oxidized in its active site. The oxidized Trx1 form is converted in the reduced form by the Thioredoxin1 reductase flavoprotein (RTrx), with the involvement of NADPH. These molecules constitute the thioredoxin redox-system1 (Trx1)

Upon reduction of target proteins, it is itself oxidized in its active site. The oxidized Trx1 form is converted in the reduced form by the Thioredoxin1 reductase flavoprotein (RTrx), with the involvement of NADPH. These molecules constitute the thioredoxin 1 (Trx1) system. Trx1 is very important for the defense of the state of health, also protecting from the tumoral pathology. Trx1 regulates the enzymatic activity, for example, of the "apoptosis signal-regulating kinase 1" [7], the caspase-3 protease that promotes apoptosis [8], and the "protein kinase C" [9]. It increases the binding and activating function on DNA [10] of different transcription factors as activator protein 1 (AP1) [11, 12], the "nuclear factor kB (NFkB) [13], the "glucocorticoid receptor" [14], and p53 [6]. Human T cells, transformed by viruses, produce a factor that is identical to the human Trx1 and that was previously called actin-depolymerizing factor (ADF) [15]. Trx1 is also secreted by activated B lymphocytes, the B lymphocytes of the type B chronic leukemia, fibroblast, and T lymphocytes [16, 17]. Trx1 is a powerful growth and survival factor [9, 12]. Its expression is increased in different types of tumor, especially in the most aggressive ones [15, 16] such as in lung cancer. In fact, increased levels of Trx1 are associated with the decrease of lung cancer patient survival. Trx1 increase has been also correlated with the inhibition of the immune system [18, 19]. Its increased expression has been identified as an independent prognostic factor of disease progression, and the expression of vascular endothelial growth factor (VEGF) and redox effector factor 1 (Ref-1) are correlated to it [20]: these are important assumptions for new therapies with monoclonal-specific antibodies for these cellular receptors.

3.3 The CD30 System

At the beginning, CD30 receptor (CD30), a member of the TNFR/NGFR family, has been identified on primary cultural cells of Hodgkin and Sternberg [21]. CD30 is also expressed on lots of other T- and B-cell lines after viral transformation; normally, peripheral blood mononuclear cells (PBMCs) express CD30 only after activation [22].

The physiological function of CD30 has not been yet clarified, but there are evidences that it could behave as a signal transducing molecule. The interaction between CD30 and its ligand (CD30L) on activated T cells, monocytes, natural killer (NK), neutrophils, eosinophils, and B cells induces the rapid activation of genic transcription factors, as JunN-kinase (JNKs) and nuclear factor NF-κB (NFkB) [23–25]. In addiction, CD30 signals induce and regulate the lymphocyte expression of cytotoxic molecules, lymphonodal traffic, proliferation, and apoptosis [22].

Advances in research have shown that CD30 is a molecule that mediates regulatory signals. These results [24–28] clarified the significance of its physiopathologic function. They showed that the interaction between CD30 and its soluble form (sCD30), released in the cell environment when CD30 interacts with CD30L, controls the physiologic homeostasis in the immune and in the neurologic systems. This is because the CD30/sCD30 interaction regulates the functions of NK, monocytes, and mature (DC) and immature (IDC) dendritic cells in order to direct the Th-cell differentiation in the respective subtypes (Treg, Th1, Th9, Th17) [24–30].

NK cells provide the first-line defense against viral infections and malignant cells. NK cells perform this important role in the immune response for their ability to kill tumor cells, for cytokine production, and for the cross-talking with the adaptive system. The cooperation with the adaptive response is mediated by the interaction between CD30 on the NK cells and CD30L on the IDC cells. This binding induces the secretion of cytokines by IDC via the mitogen-activated protein kinase pathways and promotes the differentiation of mature DC cells and the release of TNFα/IFNγ by NK cells.

At this point, it is important to highlight that from the regular development of these interactions depends the generation of DC- and Th-specific cells, a normal immune response and the protection of the health state [25].

3.4 The Functional Link Between Trx1 and CD30 Systems

Therefore, research clarified that the functional link between Trx1 and CD30 is very important for the physiologic homeostasis. Furthermore, it underlines the big potentiality of these elements as target and biomarkers in clinical treatments.

Trx1/CD30 is of key importance for Treg/Th1/Th9/Th17 cell network balance and the immune response homeostasis. In fact, the Trx1 redox system maintains balance between reduced Trx1 and oxized Trx1 which regulate, respectively, the activation/inactivation of the CD30 receptor with CD30L, modifying the stoichiometric structure of CD30 receptor (Figs. 3.2 and 3.3) [1, 31].

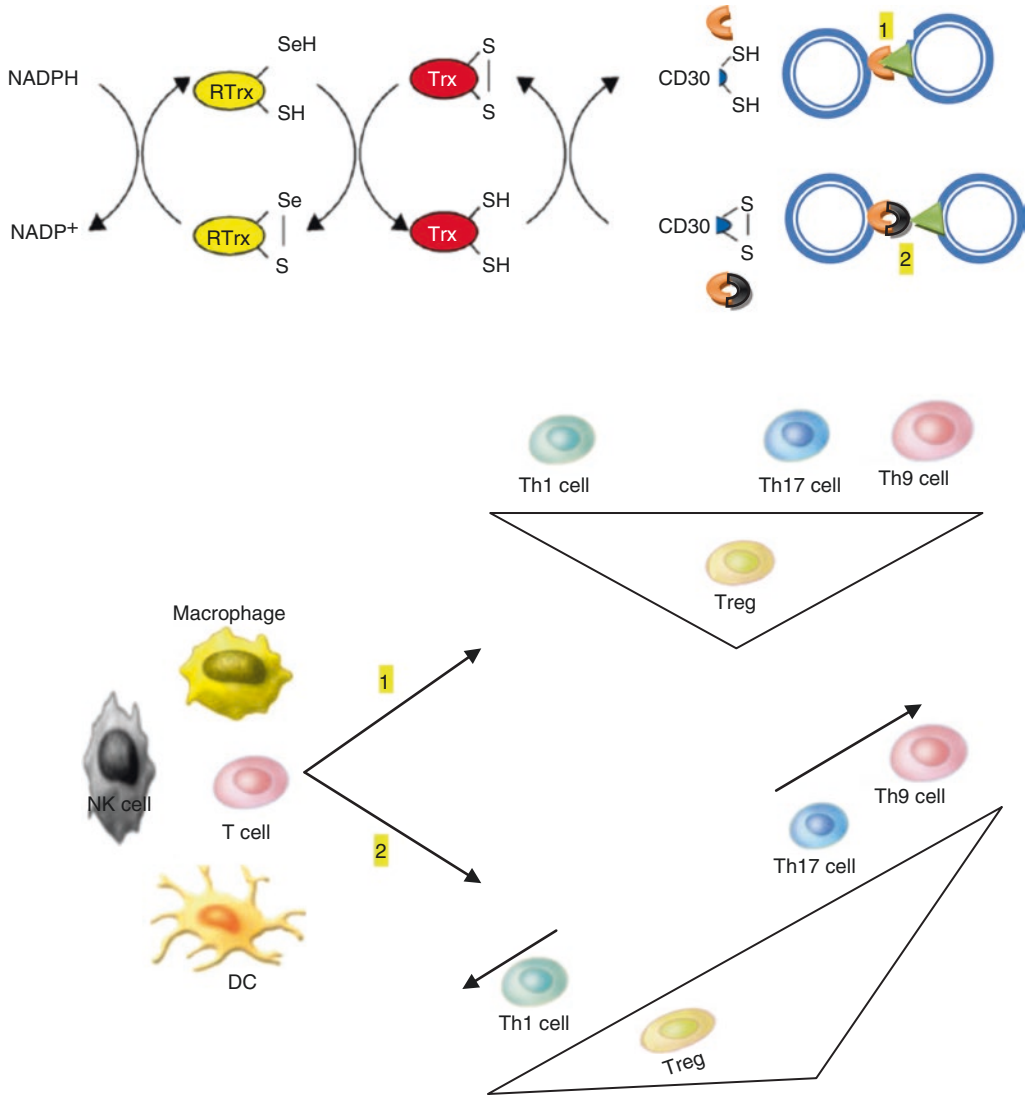

Fig. 3.2 Functional link between Trx1 and CD30 systems. Trx1 and CD30 systems regulate the Treg/Th1/Th9/Th17 network homeostasis of the immune response. The Trx1 redox-system1 maintains balance between oxidant and antioxidant Trx1, regulating the activation (1)/inactivation (2) balance of the CD30 receptor (CD30) with its ligand (CD30L). The reduced Trx1 form (Trx1-SH) is able to interact with the oxidized CD30 (CD30 S-S) and reduce it (CD30 S-H). CD30 receptor can only interact in this latter form with CD30L on activated NK, DC, monocytes, and T cells (1). On the contrary, unbalance could be the cause of non-homeostasis of the immune response and cancer development (2)

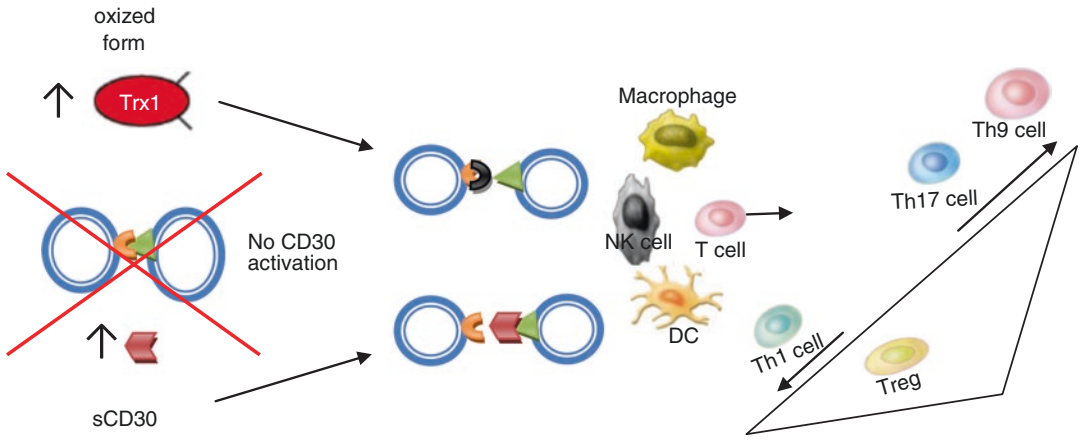

Fig. 3.3 sCD30 and Trx1 both regulate CD30R functional activation and Treg/Th1/Th9/Th17 network balance. sCD30 and Trx1 are both able to influence the CD30 capacity of mediating the activation of intracellular signals. sCD30 makes this function by binding and blocking the binding site of CD30L (◀), with which it has a strong affinity. Trx1 makes this function catalytically, modifying the stoichiometric structure of CD30. Abnormal increases in the levels of both sCD30 and Trx1oxized form result in non-activation of CD30 receptor. This causes Th9 and Th17 cell expansion and Treg and Th1 cell functional deficit, which have been noted in cancer

Furthermore, research explained that sCD30, in addition to Trx1, influences the CD30 capacity of mediating the activation of intracellular signals by CD30L. sCD30 makes this function by binding and blocking the binding site of CD30L, with which it has a strong affinity [1, 28] (Figs. 3.2 and 3.3).

The results have, also, underlined that during the inflammatory response, CD30 is largely expressed on the immune cells, and as a consequence, there is an increase of sCD30 that is released in the extracellular environment [28] (Fig. 3.3). Furthermore, it has been shown that the sCD30 level variations in the cellular or tumoral microenvironment could be used as biomarkers of the correct functioning of the immune system and the therapeutic response [1, 24–28, 32]: the sCD30 level, within the normal physiological ranges, is a positive index of the immune system homeostasis and of the therapeutic benefit. On the contrary, a significant increase of the sCD30 level is a negative index because it denotes an immunological deficit and the lack of a therapeutic response. For these reasons, both Trx1 and sCD30 have to be considered as therapeutic target.

Therefore, changes of the Trx1 and sCD30 levels are functional extracellular biomarkers of Trx1/CD30, while the Treg/Th1/Th9/Th17 cytokine levels are functional biomarkers of the intracellular pathways [1, 33–35].

These results indicate, then, that Trx1/CD30 have great potentialities to be a new double pharmacological target on which it is possible to intervene to restore the balance and the normal health state.

3.5 The Polymorphisms of KIRs, FcγRIIa-131H/R, and FcγRIIIa-158V/F Could Be Clinical Stratification Parameters to Personalize the Prognostic Trx1/CD30 Biomarkers of the Early Risk in Tumor Disease or Progression

These polymorphisms could influence the interaction between innate and adaptive immune response. In fact, as we reported above, this cooperation is mediated by the interaction between CD30/CD30L/sCD30 on NK, monocytes, DC, and IDC in order to direct the Th-cell differentiation in the respective subtypes.

It was found that only those NK cell clones expressing at least one inhibitory-specific KIR

for self-HLA class I molecule were "licensed" or functionally active. This mechanism shapes the NK repertoire and prevents NK-mediated self-damage. Thus, in tumors the downregulation of HLA class I antigen expression makes tumor cells susceptible to NK cell attack. However, often, solid tumor cells even with partial or complete loss of HLA class I expression are able to spread.

The NK cell activity is regulated by a balance of transduction signals performed by activating and inhibiting receptors [36]. The independent segregation of HLA and KIR genes, along with KIR specificity for particular HLA allotypes, makes it possible that any given individual may express KIR molecules for which there is no ligand. While gene polymorphisms encoding inhibitory KIR2DL1, KIR2DL3, and KIR2DL4 are detected in almost all individuals, those codifying for activating KIR, like KIR2DS2, are found only in a part of population. Furthermore, KIR polymorphism and its interaction with HLA alleles may influence susceptibility to inflammatory diseases, including systemic sclerosis and vascular events in systemic lupus erythematosus [37, 38], viral infections, malignancies, and pregnancy outcome [39].

Antibody-dependent cell-mediated cytotoxicity (ADCC) is, additionally, an immune defense system in mediating tumor cell killing. The FcγRs seems the only molecule on human myeloid cells capable of mediating ADCC of tumors and may be important in antibody therapy of cancer.

There are two types of FcγRs: activation receptors (CD16A and CD32A) and inhibition receptors (CD16B and CD32B) [40–42]. CD16A and CD32A activate NK lymphocytes and myeloid cells, connecting innate and the adaptive immune responses.

CD16A is expressed in NK lymphocytes and macrophages, while CD32A is widely expressed in myeloid cells [43–45]. Genes encoding for these receptors are located in the low-affinity "FCGR" locus on chromosome 1q23 [46]. FcγRIIIa gene for CD16A and FcγRIIa gene for CD32A.

Some polymorphisms of FcγR have been identified which could prove to have significant clinical relevance [43]. Two functional polymorphisms of human FcγRIIa and FcγRIIIa have been identified in the extracellular regions of these receptors: valine/phenylanine-158 of CD16A (FcγRIIIa-158V/F) and histidine/arginine-131 of CD32A (FcγRIIa-131H/R) which modulate their affinity for certain human IgG subclasses [47, 48]. Clinical studies reported that the presence of FcγRIIa-131H/H and FcγRIIIa-158V/V genotypes is associated to a more efficient ADCC antitumor response.

For these reasons, the polymorphism of KIRs, FcγRIIa-131H/R, and FcγRIIIa-158V/F has been studied as stratification parameters for the loss of the physiological homeostasis, disease risk, and its progression.

3.6 The Trx1/CD30 Double Target Is a Real Weapon to Defeat Cancer

The advances of the research have confirmed the importance of the Trx1/CD30 as double target in tumor defense. The results showed that Trx1/CD30 control the redox immunological homeostasis of the immune response both in men and women, but through different redox-immune pathways. In this control, the normal levels of Trx1/RTrx1 and sCD30 are fundamental for the preservation of IL10, TGFβ, IL4, IL6, and IL2 pathway homeostasis of immune response in the healthy subjects, also during aging. Studies in the patient groups supported this scientific rational by showing as the unbalance of the Trx1/RTrx1 and sCD30 levels generates cancer and makes it progress, through different redox-immune pathways between men and women. Then, research confirmed this role showing that the unbalance of the Trx1/RTrx1 and sCD30 levels is a biomarker of the loss of the IL10, TGFβ, IL4, IL6, and IL2 pathway homeostasis in the network of the immune response and is a risk biomarker of cancer development and progression.

Data showed also that the above redox immune unbalance is prognostic in both gender of the specific type of disease [49–59]. In men, the disease is of degenerative-destroying kind because it is

correlated to an increase of TGFβ and IL4 cytokine combination, which is a biomarker for a Th9 cell expansion [49, 50, 58, 59]. While in women, the redox-immune unbalance produces autoimmune diseases since it is correlated to an increase of the TGFβ and IL6 cytokine combination, which is a biomarker for a Th17 cell expansion [60–62]. Therefore, these and previous results [1, 52–56] showed that the susceptibility and clinical course in disease, dissimilar for genders, are caused by a different Treg, Th17 and Th9 cell polarization. This is due to the IL10, TGFβ, IL4, IL6, and IL2 cytokine pathway interactions, which vary between men and women.

The results specify, in fact, that our body produces immunological responses through physiological pathways different between men and women. However, these differences related to sex do not have consequences for the final result: the responses are activated; they perform their function and return to the initial rest phase. All this happens, normally, regardless of differences in the path between the two sexes, until there are pathological changes in these specific gender-specific pathways. In fact, if alterations occur in the pathways of IFNγ and IL6 cytokines, the effects for men and women, in terms of development of the disease, are different. This happens because in the physiological network the activity of the immune response is the result of the interactions of the activities of the entire cytokine network which is present in the microenvironment. As stated above, the cytokine pathways of IFNγ and IL6 are the main regulators of the network of the immune response of men and women, respectively. Consequently, the male gender will suffer the consequences that follow a lack of network regulation by IFNγ pathways; instead, the female sex will suffer from a lack of network regulation by the IL6 pathways.

Furthermore, it was also clarified that in these events a determining role is to be attributed to the ability of environment cytokines to activate the genic transcription factors for the differentiation of the specific Th subsets. Th1 requires the expression of Tbet transcription factor, whereas Th2 cells are controlled by expression of GATA-3 [63–65]. Treg cells differ through Forkhead boxP3 (Foxp3) transcription factor [66, 67]; instead, Th17 cells need retinoic acid-related orphan receptor gt (RORgt) [68–70], and Th9 cells need the PU.1 bet transcription factor [71–74]. There is also a mutual development relationship between Treg, Th17, and Th9 cells. TGFβ triggers the expression of Foxp3 transcription factor in naive T cells, generating Treg cells. Nevertheless, IL6 can inhibit the Foxp3 expression driven by TGFβ, and the combination of TGFβ and IL-6 cytokines is able to induce ROR-gt transcription factor, triggering the Th17 cells: nevertheless, IL2 can inhibit this induction [75]. Additionally, also IL4 inhibits induction of Foxp3 from TGFβ. The combination of TGFβ and IL4 induces the expression of PU.1 transcription factor generating Th9 cells. The co-expression of IL-9 and IL-17 was identified as a Th17 function in mediating autoimmune tissue destruction: IFNγ inhibits this generation [76].

Consequently, research has shown that Trx1/CD30 in NK, DC, monocyte, and T cells regulate the redox immunological homeostasis of the TGFβ, IL4, IL6, IL10, and IL2 gender-specific pathways. The loss of this control produces a pathological gender-specific polarization of T-cell subsets, which causes the disease development.

3.7 KIR and FcγRIIa and FcγRIIIa Polymorphisms Are Biomarkers of Low/Moderate/High Risk of Cancer Disease or Progression

The results showed that the KIR polymorphisms are stratification parameters for disease risk in healthy subjects and for its progression in patients.

The individual number of inhibitory KIR (iKIR) showed no relevance in this correlation. Instead, the number of KIR-activating receptors (aKIR) showed meaning: aKIR>2 and aKIR<3 are, respectively, biomarkers of no risk and of risk of disease and of its progression.

The increase of age is related to the increase of the disease risk, and the female gender is the

most impressed, linked to 2DS4del polymorphism. In men, the increase of risk of disease during aging is caused, primary, by the Trx1 enhance and linked to the 2DL3, 2DS4ins, and 3DL1 polymorphisms.

Furthermore, it was found that in men 3DL1 is the highest risk biomarker: it is negatively correlated with the IL2 increase and positively with the IL4 increase (prognostic for Th9 cell generation). Instead, 2DL5B is the male highest no-risk biomarker: in fact, it is positively correlated with both IL2 and IFNγ increase (prognostic for immunological response homeostasis).

As in men and also in women, 2DL5B is the highest no-risk biomarker because it is positively correlated with IL2 increase. Additionally, 2DS2/2DL2 pair is also a female no-risk biomarker: it is negatively correlated with TGFβ increase.

Results also showed that the $2DL2^+/2DS2^+$ pair is protective for tumor [77] and this is because $2DL2^+/2DS2^+$ pair is biomarker of positive interaction between innate and adaptive immunity and of immunological redox homeostasis.

Another goal of these studies is the validation of FcγRIIa and FcγRIIIa polymorphisms as gender-specific disease risk biomarkers. During aging, the FcγRIIa-131H/H combination with FcγRIIIa-158V/V is the biomarker of the lowest disease risk in both, men and women, because it is the most efficient combination for the control of redox-immune homeostasis when IL10 level is increased. The increase of IL10 level is high-risk biomarker for chronic-degenerative diseases (as tumor) and of its progression. The combinations of FcγRIIa-131H/R and FcγRIIIa-158F/F genotypes in men and of FcγRIIa-131H/R and FcγRIIIa-158V/F in women are, furthermore, biomarkers for an intermediate risk. This is because it is the most efficient combination for the control of redox-immune homeostasis when IL6 level is increased. In fact, IL6 is a pre-risk condition for the disease onset and/or its progression. The combined genotypes of FcγRIIa-131R/R with FcγRIIIa-158V/F in men and of FcγRIIa-131R/R with FcγRIIIa-158F/F in women are biomarkers for the highest risk of disease or of its progression, because they are protective only if the levels of IFNγ, IL4, and IL2 cytokines increase together. In this condition, in fact, there is no risk for the redox-immune balance.

These results showed also that in patients the combinations of H/H-F/F e R/R-V/V in men and of the H/H-V/V, H/R-V/V, and R/R-F/F in women are biomarkers of no risk of disease progression; the pair H/R-F/F is a biomarker of moderate risk only in men, while the H/H-V/F and R/R-V/F are high-risk biomarkers both in men and women; the combination H/R-V/F is a high-risk biomarker only in men.

3.8 Concluding Remarks

Therefore, research showed that the Trx1/CD30 is a gender-specific double target and biomarker of the homeostasis/non-homeostasis of the redox immune system during aging.

Homeostasis protects the state of health because it preserves our physiological ability to defend ourselves against diseases, such as cancer. On the other hand, non-homeostasis causes incapacity to defend oneself from inflammation which makes irreversible the mechanisms that generate the disease.

Consequently, the Trx1/CD30 and the selected biomarkers are a real tool for new personalized clinical strategies to defeat cancer.

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