# Breast Cancer

Breast cancer (BC), is the most common cancer of women, and its incidence is rising especially in developing countries. Global burden of breast cancer in women, measured by incidence, mortality, and economic costs, is substantial and on the increase. Worldwide, it is estimated that more than one million women are diagnosed with breast cancer every year, and more than 400,000 will die from the disease [(1)].

There is a marked geographical variation in incidence rates, being highest in the developed world and lowest in the developing countries of the third world. However, in recent years, the incidence of breast cancer has shown an alarming increasing trend [(2)]. It is estimated that 45% of the 1.35 million new cases diagnosed each year, and more than 55% of breast cancer related deaths, occur in low-and middle income countries (LMCs) [(3)]. An estimated 1.7 million women will be diagnosed with breast cancer in 2020, a 26% increase from current levels, mostly in the developing world [(4)].

In LMCs, the infrastructure and resources for routine screening mammography are often unavailable. In such countries, breast cancer is usually diagnosed at late stages, and, due to inadequate resources, women with breast cancer may receive inadequate treatment or palliative care. Many barriers are identified for breast cancer patients in LMCs which may correlate with the lower incidence and higher mortality in those countries compared to high-income countries [(1)].

According to GLOBOCAN 2008, breast cancer accounts for 38% of all new cancer cases among women living in Egypt [(5)]. In Alexandria, Egypt, the total number of cancer cases is 5010, 4662, 5064 in 2008, 2007, and 2006 respectively. The statistics of most common cancers in 2008 according to Cancer Registery Department in Medical Research Institute were as follow: 1136 from breast, 456 liver, 403 lung, and 221 bladder [(6)].

## Risk factors for breast cancer:

### (1) Age

The strongest risk factor for breast cancer (after gender) is age: the older the woman, the higher her risk [(7)].

## (2) Reproductive factors that influence breast cancer risk

- **Age at menarche**

Early age at menarche has been consistently associated with an increased risk of breast cancer. The estimated decrease in risk per five year delay in menarche is 22%. Average age of menarche in developed countries fell from around 16-17 years in the mid 19th century to 12-13 today. Good nutrition in early life reduces the age of menarche[8].

- **Age at first birth**

The younger the woman is when she begins childbearing, the lower her risk of breast cancer. The relative risk of developing breast cancer is estimated to increase by 3% for each year of delay [9]. There is evidence that the reduction in risk of breast cancer with childbirth, and higher risk with later age at first full-time birth, may be limited to estrogen-receptor-positive tumours [10].

- **Parity**

Childbearing reduces the risk of breast cancer and the higher the number of full-term pregnancies, the greater the protection. Risk of breast cancer reduces by 7% with each full-term pregnancy, and overall women who have had children have a 30% lower risk than nulliparous women. A 15% risk reduction has been shown for women with a twin birth, compared to women giving birth to a singleton [11].

- **Breastfeeding**

Breastfeeding has consistently been shown to decrease a women's risk of breast cancer with greater benefit associated with longer duration [12].

- **Age at menopause**

Late menopause increases the risk of breast cancer. Women who have undergone the menopause have a lower risk of breast cancer than pre-menopausal women of the same age and childbearing pattern [13]. Risk increases by almost 3% for each year older at menopause (natural or induced by surgery), so that a women who has the menopause at 55 rather than 45, has approximately 30% higher risk [14].

### (3) Endogenous hormones

Higher levels of endogenous hormones have long been hypothesized to increase breast cancer risk. Studies show that post-menopausal women with the highest levels of estrogen and testosterone have 2-3 times the risk of women with the lowest levels [15]. Higher levels of the hormone, prolactin, have been associated with an increased risk of breast cancer, particularly estrogen-receptor-positive tumours [16]. Having higher levels of insulin has been associated with an increased risk of post-menopausal breast cancer in women not taking hormone replacement therapy [17].

### (4) Exogenous hormones

- **Oral Contraceptives (OCs)**

The use of oral contraceptives (OCs) increases the risk of breast cancer in current and recent users, but there is no significant excess risk ten or more years after stopping use. Cancers diagnosed in women who have used OCs tend to be less clinically advanced than those detected in never-users [18].

- **Hormone Replacement Therapy (HRT)**

Women currently taking HRT have a 66% increased risk of breast cancer compared to non-users [19]. The risk increase is temporary, with risk returning to that of a never-user within five years. A woman's Body Mass Index (BMI) modifies the effect of HRT, with a stronger effect in women with a lower BMI [20]. The risk is larger for use of estrogen-progestagen therapy compared to estrogen-only [21].

### (5) Family history

A woman with one affected first-degree relative (mother or sister) has approximately double the risk of breast cancer of a woman with no family history of the disease; if two (or more) relatives are affected, her risk increases further. Risk is higher if the relative is diagnosed aged under 50 [22, 23].

It is well established that Breast Cancer 1 (BRCA 1) gene mutation and multiple BRCA 1 exons mutations play an important role in the pathogenesis of familial breast cancer in Qalubia Governorate, Egypt [24].

### (6) Breast density

Breast density is strongly and independently related to the risk of breast cancer [25]. Breast tissue is composed of fat, connective tissue and epithelial tissue. Breasts with a high proportion of fatty tissue are described as less dense. Women with the mostdense breasts have almost five times higher risk of breast cancer than women with the least dense breasts .The effect of breast density is independent of endogenous hormones. Density is affected by menopausal status, weight and number of children, but there is some evidence that the most important determinant is inherited [26].

### (7) Previous breast disease

Benign breast disease is a generic term describing all non-malignant breast conditions, some of which carry an increased risk for breast cancer while others do not. Women with proliferative breast disease without atypia have a two-fold increased risk; whilst those with atypical hyperplasia have a more that four-fold increased risk. Women with a strong family history and nonproliferative breast lesions have a 60% increase in risk of breast cancer, but there is no risk increase for women without a family history [27].

### (8) Non-reproductive lifestyle factors

- **Bodyweight**

Overweight and obesity, as measured by high body mass index (BMI), moderately increases the risk of post-menopausal breast cancer and is one of the few modifiable risk factors for breast cancer. (BMI is calculated by dividing weight in Kg by height in meters square). Overweight post-menopausal women have a 10-20% increased risk of breast cancer, and obese post-menopausal women a 30% increase in risk. In contrast, obese pre-menopausal women have a 20% reduction in breast cancer risk [28].

The link between BMI and breast cancer risk is likely to be due to hormones. In post-menopausal women, the main endogenous source of estrogen is the conversion of hormones in

fatty tissue. This is likely to explain the higher risk in overweight post-menopausal women. The reduction in risk in obese pre-menopausal women may be due to the increased likelihood of anovulatory menstrual cycles in this group [29].

- **Physical activity**

About 50 studies have looked at the association between physical activity and breast cancer, showing a 15-20% risk reduction for the most active women, with the strongest association shown for post-menopausal women [30]. The effect of physical activity on breast cancer risk may be due to how it affects hormone levels, with a recent European Prospective Investigation of Cancer (EPIC) study showing lower levels of estrogen and testosterone in post-menopausal women who reported higher levels of physical activity [31].

- **Alcohol consumption**

Epidemiological studies have consistently shown a significant association between alcohol consumption and breast cancer and a recent report from the International Agency for Research on Cancer (IARC), concluded that this association is causal [32]. Estimates of the relative risk associated with every additional drink (~ 10g of alcohol) consumed on a daily basis range from about 7-12%. This is possibly due to the higher levels of some sex hormones in the bloodstream of alcohol consumers than non-consumers [33].

- **Diet**

There has been a lot of research into the effects of dietary factors on breast cancer risk, but findings are generally inconsistent and inconclusive. The strongest evidence seems to be for fat intake: a meta-analysis of 45 studies [34], reported that higher total fat intake increased breast cancer risk by 13% while a recent cohort study showed a small but significant risk increase for higher intakes of saturated, monounsaturated and polyunsaturated fat [35]. The EPIC study showed that women who ate the most saturated fat had twice the risk of breast cancer, compared to those eating the least [36].

- **Shift work**

There is some evidence that women who do night shift work have an increased risk of breast cancer [37], and other studies show that sleeping longer reduces risk of breast cancer [38]. One theory is that disrupted or shorter duration of sleep leads to reduced levels of the hormone melatonin which has been shown to have anti-carcinogenic properties. Melatonin also suppresses the production of other hormones that have been linked to an increased risk of breast cancer. A recent study showed a 38% reduction in risk of breast cancer in women with the highest levels of the major melatonin metabolite, 6-sulfatoxymelatonin [39]. In 2007, the IARC classified night-time shift work as "probably carcinogenic to humans" [40].

- **Medical radiation exposure**

Ionizing radiation is an established risk factor for breast cancer [41]. The effect is strongly related to age at exposure, that is, the younger the woman is exposed, the greater the excess risk. Women who received diagnostic x-rays to the chest for tuberculosis or pneumonia between the ages of 10 to 29 have a three-fold increased risk of breast cancer [42, 43]. A much lower, 9%, risk increase has been shown for contralateral breast cancer in women treated with radiotherapy for a previous breast cancer (compared to women treated with surgery alone) [44].

### (9) Epstein-Bar virus (EBV) infection

Recent study indicated that EBV may play a role in the development and behavioral alteration of some aggressive BC. These studies proved the presence of the EBV genome in a considerable subset of invasive BC in Egyptian women. In the light of the new approaches in treating EBV-associated malignancies, these studies give a hope that a substantial percent of invasive BC could be treated with antiviral agents or with immunotherapy [45].

## Breast Cancer treatment

The primary treatment is surgical resection of the tumour through breast conserving surgery or mastectomy, which is effective for the vast majority of women with early breast cancer. To improve cancer control and survival, women are treated with chemotherapy, hormonal therapy and radiotherapy based on the patient and tumour characteristics such as stage, tumour grade and hormonal receptor status [46].

- **Chemotherapy**

In general, approximately half of breast cancer patients receive chemotherapy as part of their treatment [47]. The goal of this therapy is to destroy malignant cells while trying to maintain the integrity of normal cells. The difference between the amount of drug needed to induce successful tumorigenic action and the amount that produces toxicity in normal cells is small [48]. Carcinogenicity of many chemotherapy agents is partly dependent on their ability to induce mutagenic and clastogenic DNA damage, including base adducts, replication errors, strand breaks and crosslinks [49, 50]. Also, a number of studies provide evidence that chemotherapeutic agents induce apoptosis in different cell lines and tumors [51, 52].

The encouraging outcomes of chemotherapy achieved in the treatment of carcinomas in the 70s (advantage over hormonal therapy) have made it the major palliative mode of treatment of metastases as well as an adjuvant method to treat limited neoplasms [53]. The armamentarium of chemotherapeutic agents available for the treatment of breast cancer has expanded greatly over the past several decades, and complex regimens are nearly universal today [46].

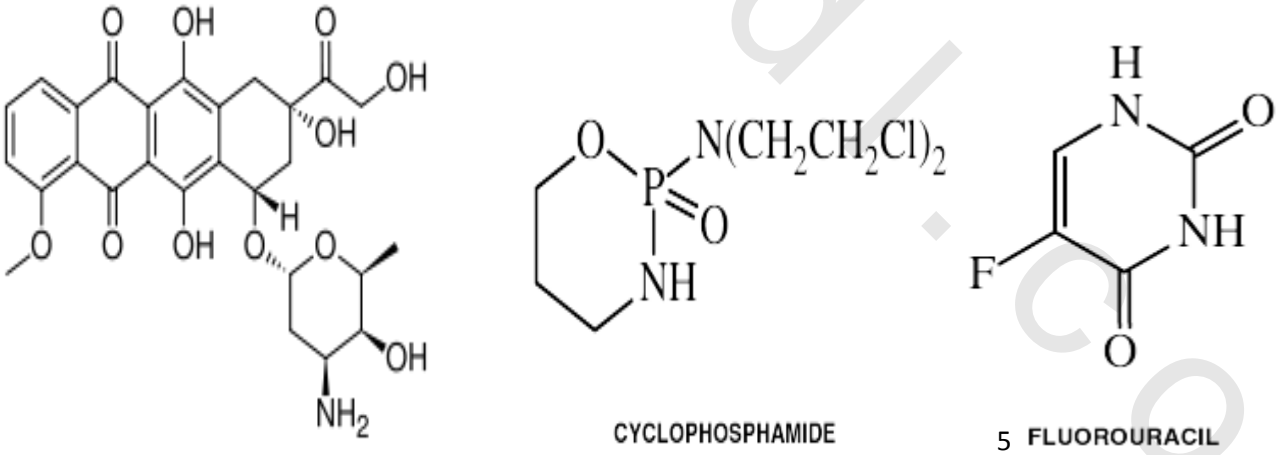
- **Anthracyclines as chemotherapeutic agents**

Anthracyclines, as integral components of most regimens, are central to the accepted treatment standards. These agents interact directly with DNA, inhibiting tumor cell proliferation and gene expression, and also lead to production of free radicals that may destroy tumor cells. Anthracyclines are important factors in optimizing adjuvant and neoadjuvant treatment and are indicated for adjuvant therapy regardless of the extent of nodal involvement, hormone receptor status, or human epidermal growth factor receptor 2 (HER-2) expression level of the tumor [46].

The most commonly used anthracyclines in breast cancer treatment are doxorubicin (Adriamycin®) and epirubicin (Ellence®). The choice of which anthracycline to use is often a function of prior experience with the agent. To optimize combination chemotherapy for early breast cancer, it is important to identify those patients who are most likely to benefit from adjuvant treatment and to understand the advantages of epirubicin over doxorubicin in specific treatment settings [46].

- **5-Fluorouracil, Adriamycin, Cyclophosphamide (FAC)**

Of several preparations that cause regression of breast cancer metastases, doxorubicin (Adriamycin) is the most active first-line monotherapeutic [54]. However, used as the second-line drug it is less effective in evoking response of proliferating neoplastic tissues. It is, however, still a golden standard in the combination of FAC (5-Fluorouracil, Adriamycin, Cyclophosphamide) [55], Fig (1). Doxorubicin is the first generation anthracycline antibiotic of wide spectrum of action. At the cellular level, it is incorporated inbetween two nitric bases of double DNA helix, thus causing the inhibition of DNA- dependent DNA and RNA polymerases [56]. This results in the suppression of DNA and RNA synthesis and damage to DNA repair mechanisms [57]. Doxorubicin also has been shown to alter topoisomerase II activity [58].




DOXORUBICIN

**Fig (1)**: FAC (5-Fluorouracil, Adriamycin, Cyclophosphamide).

Most of the currently available anti-cancer therapeutic strategies rely on the eradication of tumor cells. At first glance, approaches such as chemotherapy, radiotherapy, immunotherapy, or even oncogene targeted therapy may seem distinct. However, practically, they share the same biological mechanism in eliminating the malignant cells by programmed cell death (apoptosis). It is now increasingly accepted that part of the efficacy of anti-cancer drugs is due to their ability to activate apoptosis [(59)]. Unfortunately, the resistance of tumor cells to drug-induced apoptosis is emerging as a major category of cancer treatment failure. Therefore, amongst cancer biologists, there is increasing interest in understanding the regulatory mechanisms of apoptosis [(60)].

Among pharmacological agents, many cancer chemotherapeutic drugs are known to activate apoptotic mechanisms of tumor cell death; this may be a mechanism for current cancer treatment [(61, 62)]. Moreover, apoptosis has been proposed as a novel target for cancer chemoprevention, whose rationale is to remove cells undergoing neoplastic transformation, in situations where other defense mechanisms fail to block the carcinogenesis process upstream [(63)].

## Apoptosis

Apoptosis is a form of programmed cell death that plays a critical role in the maintenance of tissue homeostasis. It is a highly regulated physiologic mechanism that removes excess or damaged cells [(64)]. The dysregulation of apoptosis is a hallmark of cancer, with both the loss of pro-apoptotic signals and the gain of anti-apoptotic mechanisms contributing to tumorigenesis [(65)]. The induction of apoptosis in many cell types is achieved through the activation of the extrinsic and the intrinsic pathways [(66)].

**The extrinsic pathway**, Fig (2) is initiated by the interaction between specific ligands and surface receptors, such as CD95/Fas/Apo1, tumor necrosis factor (TNF) receptor 1 (TNFR1), TNF receptor 2 (TNFR2), and death receptors 3-6 (DR3-6) [(67)], which are able to deliver a death signal from the extracellular microenvironment to the cytoplasm. Binding of the ligand to the receptor induces receptor multimerization, binding of Fas-associated death domain (FADD) adapter protein, formation of the death-induced signaling complex (DISC) which recruits the initiator caspases 8 and 10, and subsequently activation of the effector caspases 3 and 7 [(67)].

**In the intrinsic pathway** Fig (2), the integrity of the mitochondrial membrane is controlled primarily by a balance between the antagonistic actions of the proapoptotic and antiapoptotic members of the Bcl-2 family [68]. This family proteins comprise three principal subfamilies:

(a) Anti-apoptotic members, including Bcl-2/Bcl-xL, which possess the Bcl-2 homology (BH) domains BH1, BH2, BH3, and BH4;

(b) Pro-apoptotic members, such as Bax, Bak, and Bok, which have the BH1, BH2, and BH3 domains; and

(c) BH3-only proteins, such as Bid, Bim, Bad, Bik, and Puma, which generally possess only the BH3 domain [68].

The Bcl-2 family of proteins regulates apoptosis by altering mitochondrial membrane permeabilization and controlling the release of cytochrome c [69].

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has led to substantial progress in understanding the multistep nature of the prolonged tumorigenesis process. This understanding has led to the realization that most human malignancies should be fought on multiple fronts. Thus, in addition to cancer therapy, cancer prevention has become an important approach to control cancer [70, 63]. Common prevention strategies include avoiding exposure to known cancer-causing agents, enhancement of host defense mechanisms against cancer, lifestyle modifications, and chemoprevention.

In fact, the induction of apoptosis is increasingly valued as a biologically significant anticancer mechanism in the arena of chemoprevention. For example, the eradication of premalignant lesions, and possibly the prevention of second primary tumors, could be achieved by chemopreventive agents that have demonstrated the capacity to trigger apoptosis in transformed cells. By shifting the mechanism of chemoprevention from cytostasis or differentiation to apoptosis, chronic exposure to a particular chemopreventive agent would not be necessary, thereby limiting the risk of long-term toxicity and/or the development of chemoresistance [70, 63].

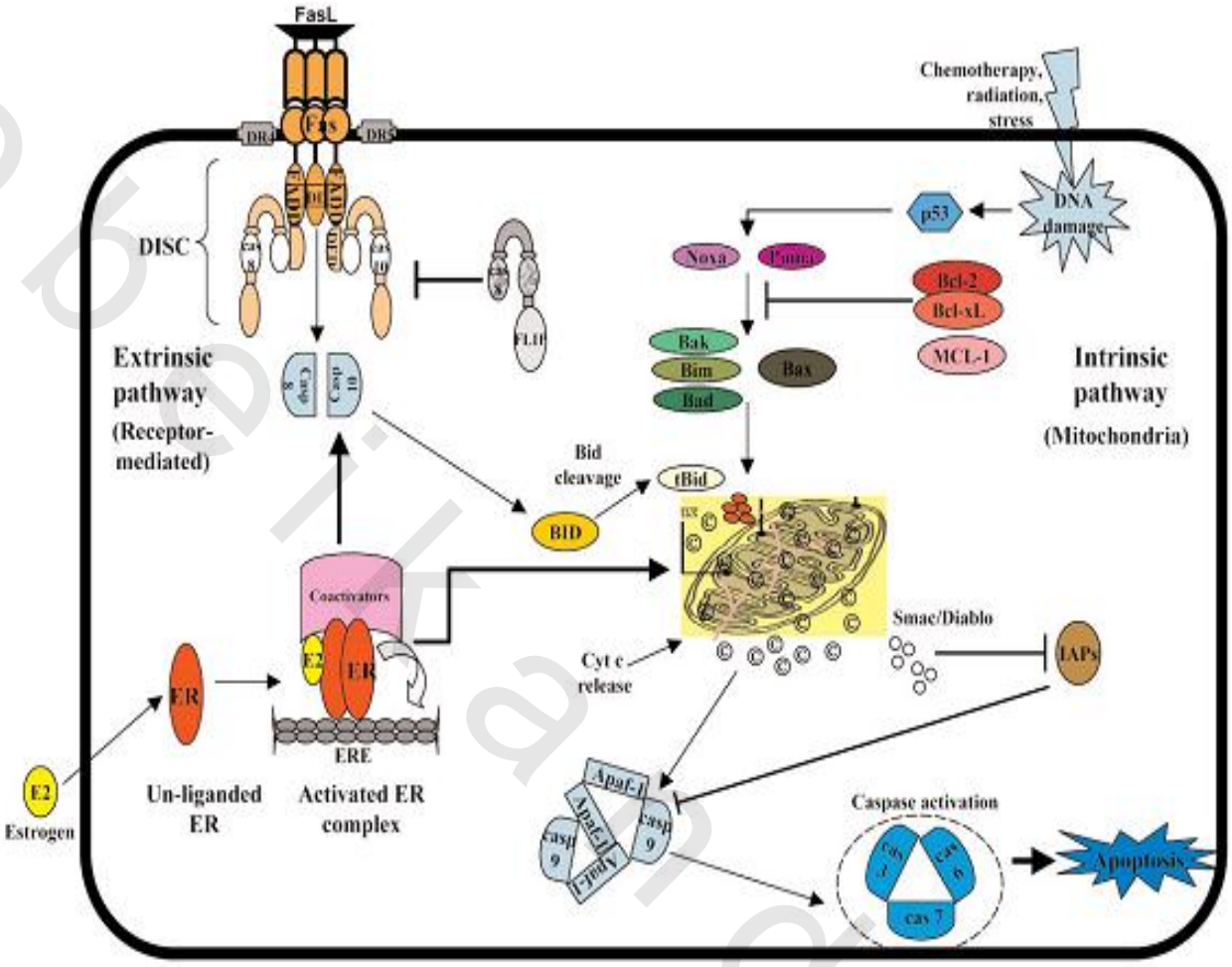



**Fig (2)**: The two main pathways involved in apoptosis regulation. (a) The extrinsic pathway begins outside the cell through the activation of receptors on the cell surface by specific molecules known as pro-apoptotic ligands, including CD95L/FasL (receptor CD95/Fas). Once activated, the death domains of these receptors bind to the adapter protein Fas-associated death domain (FADD), resulting in the assembly of death-induced signaling complex (DISC) and recruitment and assembly of initiator caspases 8 and 10. The two caspases are stimulated and processed, releasing active enzyme molecules into the cytosol, where they activate caspases 3, 6, and 7, thereby converging on the intrinsic pathway. (b) The intrinsic (mitochondrial) pathway is initiated in response to cellular signals resulting from DNA damage, a defective cell cycle, detachment from the extracellular matrix, hypoxia, loss of cell survival factors, or other types of severe cell stress. This triggers activation of specific members of the pro- apoptotic Bcl-2 protein family involved in the promotion of apoptosis, Puma and Noxa, which in turn activate the pro-apoptotic proteins Bax or Bak. These two proteins move to the mitochondrial membrane and disrupt the anti-apoptotic function of the Bcl-2 family proteins, allowing for permeabilization of the mitochondrial membrane. Apaf-1, apoptotic protease activating factor 1; Bad, Bcl-2/Bcl-XL-associated death domain protein; Bak, Bcl-2 homologous antagonist-killer protein; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2; Bcl-XL, Bcl-2-related gene, long form; Bid, Bcl-2-interacting domain; Bim, Bcl-2-interacting mediator of cell death; Casp, caspase; Cyt c, cytochrome c; E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen-responsive element; FasL, Fas ligand; FLIP, FLICE-inhibitory protein; IAP, inhibitor of apoptosis; Noxa, phorbol- 12-myristate-13-acetate-induced protein 1; Puma, p53-upregulated modulator of apoptosis.

## Tumour Suppressor Protein (P53)

Human bodies encounter mutational events on a daily basis which, if left unchecked, may lead to the development of cancer. Cellular mechanisms are therefore in place to counter this possibility. In response to various forms of stress, which may result in damage to DNA and/or proteins, the primary cellular response may be to try to repair the damage. If the damage is too severe however, then the cell may invoke mechanisms to eradicate the damaged cell via pathways of programmed cell death. A central factor controlling these processes is the tumour suppressor, p53 [(71)].

### P53 structure:

P53 is encoded by the *TP53* gene located on the short arm of chromosome 17 (17p13.1). Its sequence, about 20 Kb, contains11exons, but the first exon does not encode and is located about 10 Kb from other exons [(72)].

The protein is composed of: (i) an N- terminal region (AA 1–42), (ii) a region rich in proline residues (AA 63–97) involved in the induction of apoptosis [(73)], (iii) a core domain necessary for binding to DNA (AA 102–292), containing most of the inactivating mutations found in different types of human cancers [(74)], (iv) a tetramerization domain (AA 325–360), and (v) a C-terminal region (AA 363–393). This C-terminal region of p53 binds to the N-terminal domain of murine double minute 2 (Mdm2). In addition, there are also sequences for exporting to the cytoplasm at the N- and C-terminal ends (nuclear export signal, NES), as well as nuclear localization sequences at the C-terminal end (nuclear localization signal, NLS), enabling the regulation of subcellular localization of p53 [(75, 76)], Fig (3).

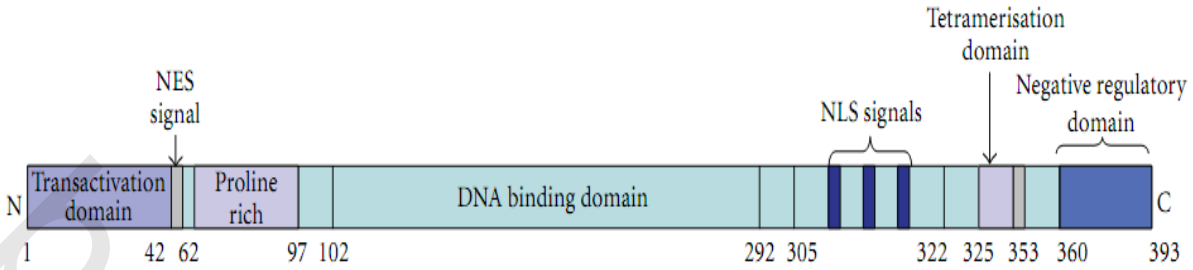



**Fig (3)**: The p53 protein structure. The N-terminal region contains the transactivation domain (AA 1-62) and a proline-rich region (residues 63-97) with a role in apoptosis. The central domain (the core domain, AA 102-292) contains specific DNA sites. The C-terminal region includes the tetramerization domain (AA 325-353) and a negative autoregulatory domain. NES signals exist on both N- and C-terminal, whereas NLS signals are located on C-terminal region.

## Stimuli and Activation Effects of TP53

Multiple stimuli such as ionizing radiations, DNA lesions, nitric oxide, hypoxia, chemotherapeutic agents, or oncogenic stimuli can activate p53 [77, 78]. In response to activation it could induce different effects. P53 is a transcription factor involved in the control of G1/S and G2/M phase transition, in DNA repair, and in induction of senescence, apoptosis, autophagy, mitotic catastrophe, and angiogenesis [78], Fig (4).

### (1) Cell Cycle Regulation.

TP53 regulates the control of the G1 checkpoint and can induce an arrest of the cell cycle, repair or apoptosis if DNA lesions are extensive [79]. Wild-type p53 protein can transcriptionally transactivate p21Cip1, a potent inhibitor of most cyclin-dependent kinases, involved in the cell cycle arrest [80]. P53 also stimulates the expression of the 14-3-3σ protein that sequesters the cyclin B1/CDK1 complex to block the transition G2/M. But p53 also induces the expression of many others genes such as GADD45, which interacts with PCNA to inhibit the passage to S phase, or Reprimo to block the cell cycle in G2 phase [81].

**(2) Cell Senescence.**

Cellular senescence is thought to play an important role in tumor suppression and to contribute to cellular aging [82]. The p53 tumor suppressor is also a critical mediator of senescence, and it seems to play a critical role in the induction and maintenance of cellular senescence. P53 activation is an essential step in the induction of senescence following DNA damage or other forms of stress. In the context of senescence, p53 is controlled by different proteins which cause the posttranslational stabilization of p53 through its phosphorylation [83].

**(3) Apoptosis.**

Apoptosis is one of the principal functions of p53. It has been shown that p53 can transactivate the cell death receptors CD95 or TNF which induce the formation of the DISC complex and finally activate caspase 8. P53 also activates proapoptotic members of the Bcl2 family: Bax, Noxa, and Puma-involved in the permeabilization of the outer mitochondrial membrane [84]. Moreover, p53 has also been reported to have a direct role in cell death initiation by localizing to mitochondria and regulating mitochondrial outer membrane permeabilisation directly. Thus, p53 protein can directly induce permeabilisation of the outer mitochondrial membrane by forming complexes with the protective BclXL and Bcl2 proteins, resulting in cytochrome c release [85, 86].

**(4) Angiogenesis**

The formation of new blood capillaries (angiogenesis) is closely regulated by proangiogenic and antiangiogenic factors [87]. The p53 protein has been shown to limit angiogenesis by few mechanisms: (1) interfering with central regulators of hypoxia that mediate angiogenesis, (2) inhibiting the production of proangiogenic factors, and (3) directly increasing the production of endogenous angiogenesis inhibitors. The combination of these effects allows p53 to efficiently shut down the angiogenic potential of cancer cells [88]. Wild-type p53 plays a role in limiting tumor vascularization as demonstrated by some clinical studies [89].

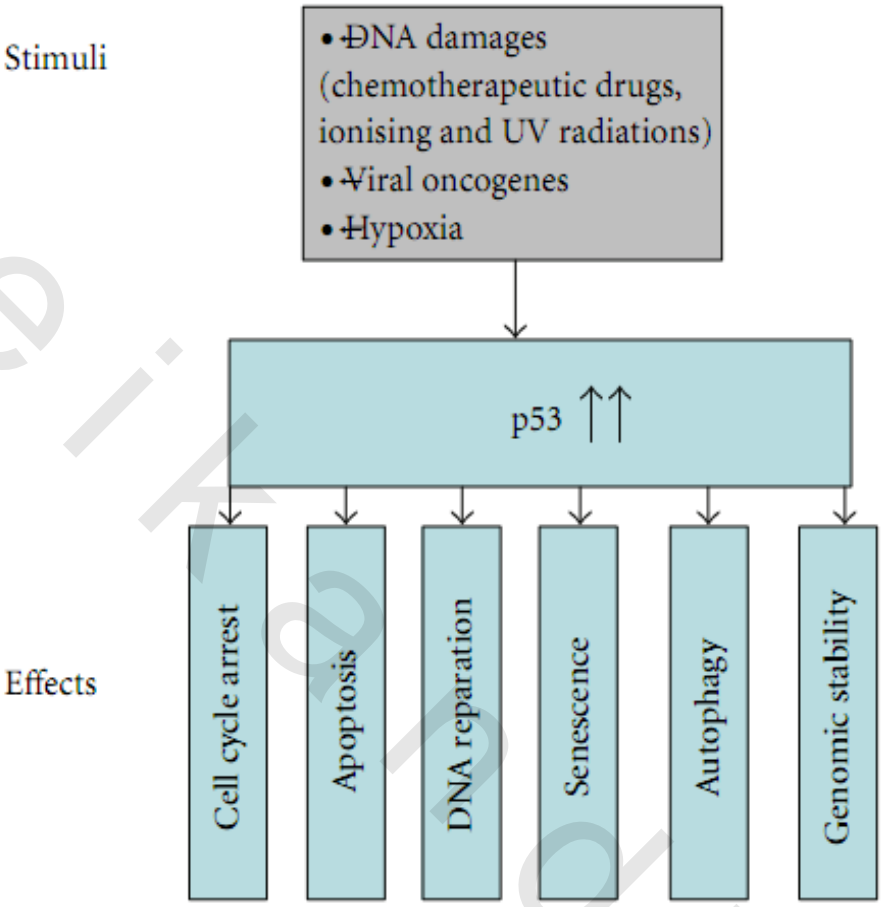



**Fig (4)**: Stimuli and effects of activation of p53 protein. In response to diverse stimuli p53 functional protein induces diverse effects such as cell cycle arrest, apoptosis, repair of DNA lesions, senescence, and autophagy.

**Regulation of p53**

The protein p53 can be regulated at different levels: (i) by posttranslational modifications, such as phosphorylation, or acetylation of the protein [90, 91], (ii) by increasing the protein concentration: one of the key regulators of p53 is Mdm2 which targets p53 for breakdown by the proteasome [92], (iii) by cellular localization: import and nuclear export is closely regulated because the functions of p53 depend on its nuclear localization. Efficient transfer to the cytoplasm depends on Mdm2 forming a complex with p53, which is why ubiquitin ligase activity of Mdm2 is essential for nuclear export of p53.

**Human Cancers and p53 Mutations.**

More than 26,000 somatic mutation data of p53 appear in the IARC TP53 database version R14 [94]. The frequency of TP53 mutation varies from about 10% (hematopoietic malignancies) to 50–70% (ovarian, colorectal, and head and neck malignancies) [95]. Germ line mutation of TP53 causes Li-Fraumeni syndrome, which is a familial cancer syndrome including breast cancer, soft tissue sarcoma, and various other types of cancer [96]. Most TP53 mutations in human cancers result in mutations within the DNA-binding domain, thus preventing p53 from transcribing its target genes. However, mutant p53 has not only led to a loss of normal function of the wild-type protein but also led to new abilities to promote cancer [95].

## The human inhibitors of apoptosis protein (IAP)

Resistance to apoptosis contributes to carcinogenic transformation of cells and tumourigenesis as well as to malignant progression of tumours and lack of their responsiveness to non-surgical modes of therapy [65, 97, 98]. The human inhibitor of apoptosis protein (IAP) family encompasses eight members. These proteins participat6e in the regulation of a variety of cell functions including apoptosis, cell division, non-apoptotic signal transduction and copper homeostasis. IAPs block apoptotic cell death primarily via mechanisms that repress the activation and activity of certain caspases and/or reduce their protein levels [99, 100].

## Survivin

Survivin, is a small 16.5 kDa protein belonging to the inhibitor-of-apoptosis (IAP) family [101]. Interest in survivin stems from its pattern of expression: it is upregulated in almost all tumour types while being minimally expressed in terminally differentiated tissues [102]. Moreover, it plays a key role in both apoptosis and control of cell-cycle progression, and in tumours its expression is associated with poorer prognosis and increased treatment resistance [103]. It is a multifunctional protein which controls cell proliferation, inhibition of apoptosis, and the promotion of angiogenesis. Gene encoding survivin is among the top 5 most tumor specific genes in human genome based on comparisons of the number of times survivin transcripts appear in tumors compared with normal cells and tissues. This makes survivin as one of the top “transcriptomes” that are expressed in cancer but not in normal tissues [104].

Although the over-expression of multiple IAPs has been implicated in apoptosis resistance of human neoplasms [100, 105-108], recent data indicate that high expression of both survivin and XIAP is particularly critical for apoptosis suppression in cancer cells derived from solid human tumours [109-114]. Moreover, survivin and XIAP are also upregulated in angiogenic factor-activated endothelial cells of tumour microvasculature and protect them from endogenous and exogenous apoptotic stimuli [115-118].

## Structure and Function of Survivin

The smallest member of the IAP family, survivin is composed of a single baculovirus IAP repeat (BIR) domain and an extended α-helical coiled-coil domain at the carboxy terminus [119], Fig (5). It functions as a homodimer, requiring the BIR domain for dimerisation and recruitment of other proteins such as caspase 3, p21 and Cdk4 [120].

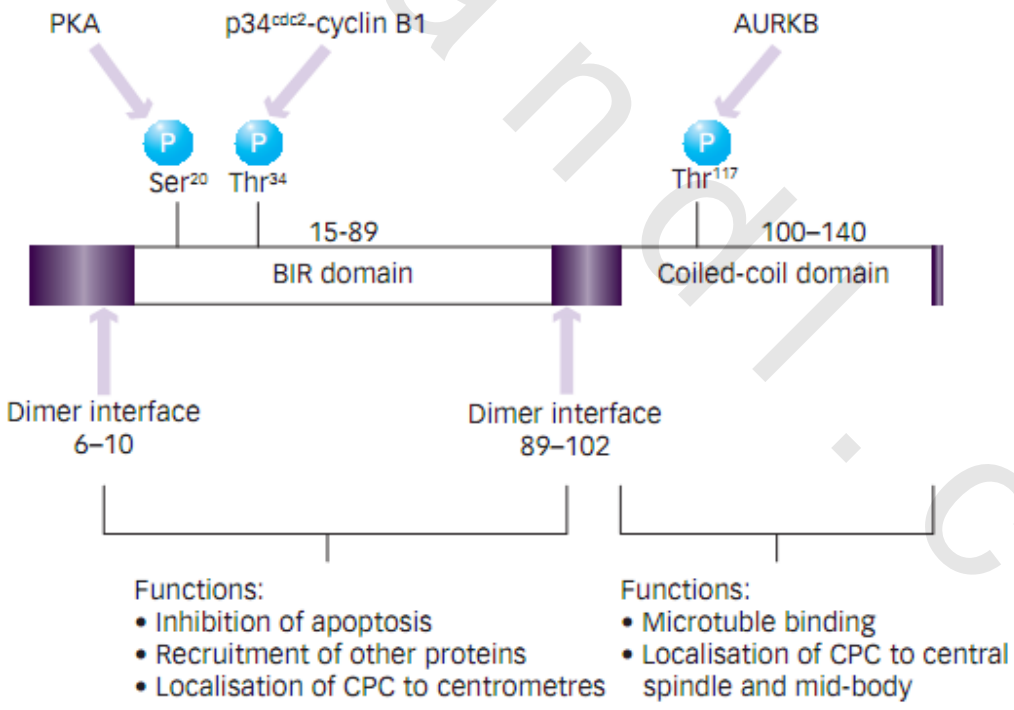



**Fig (5)**: Survivin Protein Structure and Function.

**Localization of survivin**

In normal tissue survivin is not seen in mitochondria suggesting that it is exclusively associated with tumor transformation. Survivin subcellular compartmentalization in mitochondria seems to play role in antiapoptotic function of protein [121]. In response to cell death stimulation, survivin is rapidly discharged and released in the cytosol where it prevents caspase activation thus inhibiting apoptosis. Survivin shuttles between nucleus and the cytoplasm and hence can associate with different subcellular components. Splicing studies have shown that nuclear staining of survivin is associated with favorable prognosis but the cytoplasmic survivin expression correlates with tumor stage and histological grade and not with cancer prognosis [122].

**Survivin in body fluids**

Survivin and anti survivin antibodies are detected in serum from breast cancer patients. In a study of 67 patients with breast cancer and 135 healthy women, survivin mRNA concentrations in the peripheral blood was found to range from 0 to 3.32 pg/ml with a median of 1.03 pg/ml, while in healthy person it ranges from 0 to 0.54 pg/ml (median 0.192 pg/ml) [123]. Another study showed no difference in the serum and urine survivin levels in 43 breast cancer patients and controls, similarly no statistically significant effect of chemotherapy on serum and urine survivin levels were observed [124].

**Survivin and Cancer**

Survivin is almost undetectable in most adult tissues, and expression is largely limited to developing embryos and haematopoietic, epithelial and gonadal cell lines, where expression is often cell-cycle-dependent [102]. However, survivin over-expression has been reported in nearly all human malignancies [125]. Because of this upregulation in malignancy, and its functional involvement in apoptosis as well as proliferation, survivin is currently attracting considerable interest both as a potential cancer biomarker [126] and as a new target for cancer treatment [127]. Overexpression of survivin renders cancer cells resistant to anti-cancer therapy including chemotherapy and radiation therapy [128-132]. Overexpression of survivin inhibited the tamoxifen and cisplatin-induced apoptosis of human breast and gastric cancer cells [130, 132].

The level of survivin expression was inversely related to the degree of apoptosis and positively related to the risk of local tumor recurrence in rectal cancer patients treated with radiotherapy [133]. Patients with gastric tumors that express low levels of survivin appear to have a longer mean survival time after cisplatin treatment than patients with high levels of expression [132]. Survivin expression is associated with the metastasis of human prostate cancer to bone. Thus, survivin plays an important role in tumorigenesis and tumor metastasis, where levels of survivin expression serve as an indicator of therapeutic effectiveness [134].

**Inhibition of apoptosis**

Multiple *in vitro* and *in vivo* studies have shown that survivin inhibits cell death, especially apoptosis. *In vitro,* survivin was found to counteract both intrinsic and extrinsic mediators of apoptosis, including IL-3 withdrawal, FAS stimulation, TRAIL, over expression of BAX, p53, caspase-3, -7, and -8, methotrexate, taxol and TNFa/ cyclohexamide [101, 135-140]. In certain cell types however, the level of inhibition was less potent than that achieved with BCl-2 or other IAP members [136, 141]. Similarly, survivin has been shown to block apoptosis *in vivo* [142, 143].

The ability of survivin to counteract apoptotic stimuli enhances cell survival, which in turn facilitates cell proliferation, including the proliferation of mutant cells. This proliferation may ultimately give rise to malignancy. The failure to execute apoptosis also renders malignant cells resistant to various forms of therapy including chemotherapy and radiotherapy [144].

Although convincing evidence exists that survivin inhibits apoptosis, the exact mechanism by which this occurs is incomplete. As with other IAPs [145], the interaction between survivin and initiator or effector caspases has been reported by several groups, [146,147] and a physical proximity between survivin and caspase-3 has been described at the pericentriolar area [148]. However, while some studies reported that this contact resulted in inhibition of activity [149, 150], others found that it did not translate into meaningful inhibition of caspase activity *in vitro* [151].

Indeed, a recent report found that chemically synthesized survivin could not inhibit caspase-3, even at very high concentrations [152]. The current thinking therefore is that most IAPs, including survivin, block apoptosis by mechanisms other than of direct effector caspase inhibition [127, 153]. Like most other IAPs, it acts upstream of effector caspases rather than through direct caspase

inhibition [125]. In the cytosol, survivin associates with hepatitis B X-interacting protein (HBXIP) and the complex binds to pro-caspase 9, preventing its recruitment and activation by the apoptosome [154]. Another mechanism involves the sequestration of second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO).

Smac/DIABLO is a mitochondrial pro-apoptotic signal that binds X-linked IAP (XIAP), thereby preventing apoptosis inhibition. Survivin has been shown to prevent the release of Smac/DIABLO from the mitochondria, leaving XIAP free to inhibit caspase activity [155]. Lastly, survivin can also bind directly to XIAP, stabilising it against degradation and resulting in inhibition of apoptosis [156].

**Regulation of Survivin**

The survivin gene is regulated by a number of transcription factors including β-catenin, activated T-cell factor (TCF), signal transducer and activator of transcription (Stat)-3, hypoxia-inducible factor-1 alpha (HIF-1α), and heat shock protein (Hsp) 90 [157]. Factors upstream also play an important regulatory role. One study found that in normal melanocytes, p53 and retinoblastoma (Rb) are required to repress survivin transcription, with Rb exerting its effects through E2F and p53 repressing Sp1-mediated expression [158]. Post-translational modifications also play an important regulatory role. Phosphorylation of Thr34 by p34-cdc2-cyclin B1 slows down survivin clearance by the proteasome [157]. Phosphorylation of Thr117 regulates survivin's cell-cycle activities, while phosphorylation of Ser20 (by protein kinase A [PKA]) facilitates the interaction of survivin with XIAP [156, 159]. Several sites of ubiquitination have also been identified and these include Lys23, Lys62, Lys78 and Lys79 [127]. Finally, Hsp90 acts as an important chaperone for survivin, necessary to prevent proteasomal degradation [160].

## Circulating DNA

Cell-free circulating DNA (CFDNA) has been studied in a wide range of physiological and pathological conditions, including pregnancy, trauma, inflammatory disorders and malignancy [161, 162]. It is present in normal healthy individuals at low concentrations (ng/ml) [163]. Raised levels of CFDNA in cancer patients have been reported in many tumour types [162-164]. The finding that genetic and epigenetic changes typical of tumours can be detected in CFDNA from

cancer patients suggests that the excess CFDNA is of tumour origin. It probably derives from a combination of apoptosis, necrosis and active release from tumour cells [165]. Such cell-free DNA has shown promise for improving early clinical diagnosis, prognostication and disease monitoring in inaccessible tumour types, such as lung cancer [166-170].

**Sources of circulating DNA:**

The presence of high levels of circulating DNA in blood of tumor patients has been suggested to be caused by apoptosis and necrosis of tumor cells, or release of intact cells into the blood stream and their subsequent lysis [162, 165, 166]. Correlations between the occurrence of cell-free DNA in blood of tumor patients and malignancy of their disease were reported [171, 172]. Based on the low DNA levels in blood of healthy controls and the high cellular turnover in tumors, the circulating DNA fragments might originate from a primary tumor, which discharges its DNA into the blood circulation [171]. The clearance of cell-free DNA from the bloodstream occurs rapidly: fetal DNA disappeared from the blood of mothers after delivery with a half- life time of 16.3 minutes [173]. It is known that cell-free DNA is sensitive to plasma nucleases (e.g. DNase 1), but renal, and hepatic clearance are also involved in the elimination of cell-free DNA (cfDNA) [174, 175].

**Apoptosis as source of cfDNA:**

DNA fragmentation is the hallmark of apoptosis [176-178]. Apoptosis has a distinctive DNA ladder pattern was observed in agarose gels that showed specific banding at 200 bp and multiples thereof. The banding pattern was apparently due to endonuclease-mediated double-strand cleavage between nucleosomes [179].

Three distinct steps of DNA degradation, likely mediated by different enzymes, can be identified as follows: (i) at relatively high (2 mM), $Mg^{+2}$ concentration DNA is fragmented to 0.05–1 Mb size sections (type-I; so called high molecular weight DNA fragmentation); (ii) at nanomolar $Ca^{+2}$ concentration, DNA is cleaved into 300 Kb fragments (type-II; intermediate DNA fragmentation); and (iii) at the micromolar levels of $Ca^{+2}$, internucleosomal (type-III) DNA fragmentation takes place leading to formation of DNA sections of the size of mono and oligonucleosomes, which can be detected by a characteristic ‘‘DNA-ladder’ pattern during electrophoresis [177, 178].

Amongst the nucleases involved in DNA fragmentation during apoptosis the best characterized is Caspase-Activated DNase (CAD), with its inhibitor ICAD (Inhibitor of CAD) in mice, and its homologue DFF40/DFF45 (DNA Fragmentation Factor) [180] in human. CAD (or DFF40) remains inactive in a complex with its respective inhibitor until cleavage of the inhibitor by activated caspase leads to its activation. Upon activation it undergoes translocation into the nucleus where it targets internucleosomal DNA. In some human tissues, DNase-X appears to substitute of DFF40 [181]. Also involved in DNA fragmentation during apoptosis is DNase-I [182], which is activated by $Ca^{+2}$ and $Mg^{+2}$ and inhibited by $Zn^{+2}$ [183, 184]. DNase-I is localized in the perinuclear space [185], and if over expressed induces apoptosis [186]. Among other nucleases linked to DNA fragmentation during apoptosis are DNAS1L3 [187, 188], NUC18 [189], and NUC70 [190], all activated by $Ca^{+2}$ and $Mg^{+2}$ and inhibited by $Zn^{+2}$. The acidic endonucleases DNase-II [191] and L-DNase-II [192] also appear involved in apoptotic DNA fragmentation. The protein released from the inter-membrane compartment of mitochondria named apoptosis inducing factor (AIF) has been also reported capable of inducing chromatin condensation and DNA cleavage into megabase-size fragments [193].

**Necrosis as source of cfDNA:**

Cell necrosis may be another possible mechanism of DNA fragmentation, because necrotic cell death is significantly less prevalent than apoptosis in a healthy body. Necrotic cells are generally not found in the normal tissue, often observed as a result of insufficient vascularization and persistent ischemia. Tumor necrosis is a frequent event in solid malignant neoplasms, but it generates a spectrum of DNA fragments with different strand lengths because of random and incomplete digestion of genomic DNA by a variety of DNAases [194, 195].

**Quantification of cfDNA:**

The development of improved detection methods led to the discovery of increased circulating DNA levels in cancer patients compared to healthy individuals [196]. It was later confirmed that cancer patients' DNA levels are not only increased compared to healthy individuals, but also to patients with various non-malignant diseases [197]. Finally, it was shown that the circulating DNA carried tumor-specific alterations [198]. The amount of circulating DNA is within the range of nanograms, and thus the detection of circulating DNA was laborious until the development of the

polymerase chain reaction (PCR). After development of PCR and quantitative PCR techniques, knowledge of cell-free circulating DNA was rapidly growing: It was shown for many tumor entities that cell-free circulating DNA levels are increased and allow distinguishing patients with non-malignant disease from healthy individuals (e.g. lung [164], colon [199], cervical [200], ovarian [201], breast [202], testis [203], bladder [204] and prostate cancer [205]). Cell-free DNA may therefore serve as a non-invasive universal cancer biomarker.

**DNA Integrity:**

It is believed that apoptotic cell death is the predominant source of cDNA in healthy individuals, producing short, uniform DNA fragments. In contrast, DNA released from malignant cells varies in size [206] therefore, elevated levels of long DNA fragments may be a good marker for detection of malignant tumor DNA in blood [165]. DNA integrity specifically represents the relative amount of nonapoptotic cell death [207].

**DNA integrity**: is identified as the presence of DNA fragments in the blood with variable lengths or sizes > 200 bp [195].

**DNA integrity index**: is defined as the ratio of longer fragments "> 200" to shorter fragments "<200" [195].

DNA integrity and DNA integrity index may vary between healthy individuals and cancer [208]. An increased level of large/necrotic DNA fragments was associated with advanced tumor stage in breast cancer patients [206]. Therefore cDNA lengths may represent a useful novel biomarker for the diagnosis, prognosis and surveillance of patients with breast cancer [209].

**Possible explanation of DNA integrity:**

Because one of the characteristics of apoptosis can be the cleavage of DNA into 180 to 210 fragments [210], the presence of high-molecular-weight fragments detected by the examination of DNA integrity in cancer patients may indicate that a certain population of cells has escaped apoptosis-induced DNA degradation [211]. DNA integrity analysis may be useful method for detecting the abrogation of apoptosis. Furthermore, it may be useful for staging and monitoring of treatment of different cancer types, where abrogation of apoptosis has been indicated as an intrinsic factor in disease progression [211-214].

## Cytochrome C ( Cytc)

Cytc is an evolutionarily conserved nuclear-encoded mitochondrial protein, which contains 104 amino acids in mammals. It is highly positively charged with a pI of 9.6. Cytc is essential for aerobic energy production and Cytc knockout mice die around midgestation [215].

### Functions of Cytc

Cytc is a multi-functional enzyme that is involved in life and death decisions of the cell Fig (6). It participates in electron transfer as part of the mitochondrial electron transport chain (ETC) and is thus an indispensable part of the energy production process. It is also essential for the formation of the apoptosome and the progression of apoptosis. Recent discoveries of additional functions of Cytc, including its activity as a cardiolipin peroxidase [216, 217], and the detection of four phosphorylation sites on Cytc, suggest that its multiple functions are regulated by cell signaling pathways. Derivation of the specific pathways that operate these regulatory mechanisms and their effects may become an important avenue for therapeutic targeting of various human illnesses including neurodegenerative diseases, congestive heart failure, and cancer [218-220].

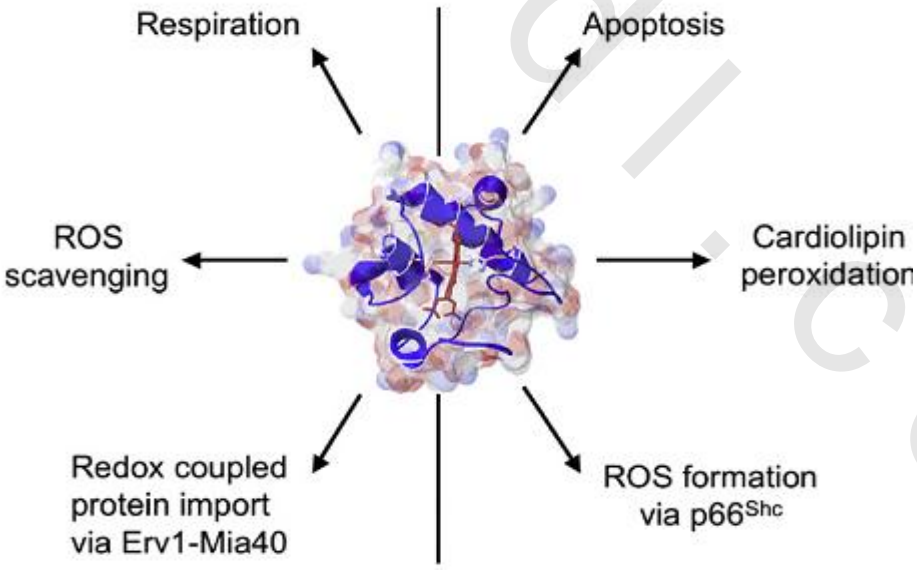



**Fig (6)**: Summary of the main functions of cytochrome c. The left side of the figure highlights its involvement in life-sustaining functions, whereas its involvement in cellular death functions is shown on the right side.

**Cytc and apoptosis**

It is now generally accepted that a key step in the apoptotic cascade involves the release of Cytc into the cytosol where it binds with apoptotic protease-activating factor 1 (Apaf-1). Binding of Cytc results in an increased affinity of the complex for dATP whose binding is necessary for oligomerization and formation of the apoptosome [221]. The apoptosome recruits multiple pro-caspase-9 molecules and promotes their cleavage to an active form, known as the initiators of apoptosis. Caspase-9 bound to the apoptosome acts as the cleavage factor of caspase-3, or what is considered the major enzyme in the committal to apoptosis [221, 222]. Other studies have suggested that Cytc release initiates apoptosis by binding inositol trisphosphate (IP3) receptors, causing calcium release into the cytosol [223], and subsequent calpain activation and apoptosis-inducing factor (AIF) release [224].

The intrinsic pathway of apoptosis is mediated by various stimuli that cause the release of Cytc from mitochondria into the cytoplasm, triggering caspase activation [222, 225]. Though the release of Cytc from mitochondria is irreversible, previous evidence suggests that the execution phase of apoptosis is highly regulated even after Cytc release [226, 227]. There are several possible levels of such regulation and the redox state of cytosolic Cytc may be one of them [228].

**The apoptosome**

Once released from mitochondria, Cytc participates in assembling a multimeric, caspase-9-activating complex — the apoptosome. Studies on reconstituted apoptosomes using purified protein indicate that Apaf-1, Cytc, pro-caspase-9 and dATP/ATP are the necessary and sufficient components of the complex, though some additional proteins may be involved (such as XIAP, Hsp70 and Aven) [229,230]. Apaf-1 is a cytosolic protein existing as an inactive, monomeric, closed conformation until the apoptotic signal– appearance of Cytc in cytosol – is received. Cytc binds to the WD40 domains of Apaf-1, stabilizing an open conformation of Apaf-1 that hydrolyses dATP or ATP bound to the nucleotide-binding domain. This is followed by nucleotide exchange, which triggers oligomerisation into a heptamer of 7 Apaf-1molecules, which is then capable of recruiting and activating pro-caspase-9 via the CARD domains of Apaf-1 and pro- caspase-9 [231].

**Mechanisms by which the redox state of Cytc may regulate apoptosis, Fig (7):**

Apoptosis can be regulated by many different redox molecules acting at different sites in the cell [228, 232, 233]. It is possible that once Cytc is released into the cytoplasm it comes into equilibrium with the 'cytosolic redox state' i.e. the system of oxidants and antioxidants that are broadly in equilibrium with reduced/oxidized glutathione. However, it seems more likely that reduction and oxidation of Cytc in the cytoplasm is dominated by specific enzyme catalysed reactions, such as that of cytochrome oxidase.

Experimental evidence indicates that the redox activity of Cytc is not necessary for apoptosome formation or activation of caspases (i.e. it does not have to CHANGE redox states). However, there remain at least three possible mechanisms by which the redox state of Cytc might influence activation of the apoptosome:

(a) The reduced and oxidized forms of Cytc might have different binding affinities for Apaf-1. To be consistent with the results above, the reduced form of Cytc would need to have a lower affinity or on rate for binding to Apaf-1. Binding of Cytc to Apaf-1 involves many residues on different areas of the surface, particularly lysine residues around the haem edge [234, 235], and the relative positions of these residues does change with redox state, but as the binding is mainly electrostatic it is hard to know whether this will have much effect on the affinity.

(b) The reduced and oxidized forms of Cytc might have different abilities to activate Apaf-1 (after binding). Binding of Cytc to Apaf-1 causes hydrolysis of bound dATP to dADP, followed by nucleotide exchange with free dATP, which then allows activation of the apoptosome, whereas if the nucleotide exchange does not occur the apoptosome becomes irreversibly inactivated. The reduced form of Cytc might bind to monomeric Apaf-1, but it incapable of causing dATP hydrolysis or exchange, thus blocking activation, and potentially leading to inactivation.

(c) Reduced cytochrome c might be less capable of activating the apoptosome because it preferentially binds to other cell components (proteins, membranes, DNA) thus leaving less free to bind to the apoptosome. Cytc has a net positive charge and is known to bind to DNA, to negatively charged membranes, and to a variety of cytosolic proteins (such as cytochrome b5 and the IP3 receptor). The redox state of Cytc might affect the affinity of binding to these

components. Cytc also binds to ATP and this is known to block its activation of Apaf-1, so if the reduced form of Cytc bound more ATP this might explain its inability to activate [236].

The redox state of cytochrome c might also regulate apoptosis upstream or downstream of caspase activation. According to the work of Kagan and his coworkers in [217, 237], the oxidized form of Cytc can peroxidize mitochondrial cardiolipin, which apparently favours Cytc release from the mitochondria. Since the reduced form of Cytc is unlikely to be capable of oxidizing cardiolipin, this might be one way in which the redox state of Cytc regulates apoptosis, although such regulation would be prior to Cytc release. Kagan's group have also provided evidence that once released from mitochondria the oxidized form of Cytc can peroxidize phosphatidylserine on the inside of the plasma membrane. This oxidized form of phosphatidylserine is apparently preferentially exported to the outer leaflet of the plasma membrane, where it provides an 'eat-me' signal to phagocytes. Here again, since the reduced form of Cytc is unlikely to be capable of oxidizing phosphatidylserine, this might be one way in which the redox state of Cytc regulates apoptosis, although such regulation would be at the level of phosphatidylserine exposure [216, 238].

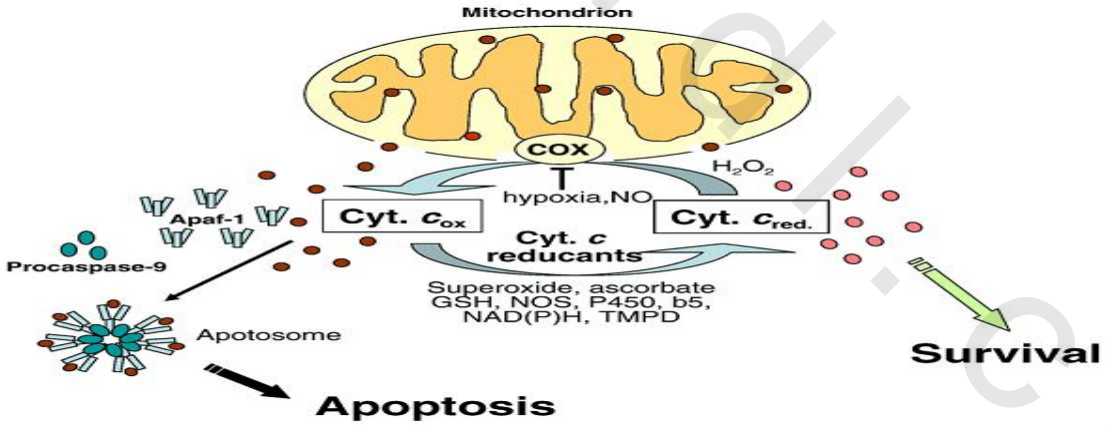



**Fig (7)**: Regulation of apoptosis by the redox state of cytosolic cytochrome c. Cytochrome c is oxidized by mitochondrial cytochrome oxidase (COX) and in this oxidized form (Cytc ox) it binds to Apaf-1 forming the apoptosome which activates pro-caspase-9 leading to apoptosis. Cytosolic cytochrome c can be reduced (Cytc red) by various reductants which include superoxide, ascorbate, reduced glutathione (GSH), some chemicals such as tetramethylphenylenediamine (TMPD) and reducing enzymes (cytochromes b5, P450, NOS, neuroglobin, cytosolic NAD(P)H oxidases). This reduced cytochrome c cannot activate the apoptosome, and therefore does not promote apoptosis .

## Vitamin supplementations and breast cancer treatment

There is a considerable number of *in vitro* and animal data showing that vitamins and other antioxidants can protect cells against radiation and chemotherapy. It is also sometimes claimed that antioxidants are directly cytotoxic and/or can actually increase the effectiveness of cytotoxic treatments. For example, *in vitro* studies have reported that vitamins A, C, and E, as well as carotenoids, can enhance the effectiveness of chemotherapy [(239)]. Of great interest is the potential of drug–drug or drug–antioxidant combinations to act in an additive or synergistic fashion. Synergistic drug–drug and drug–antioxidant interactions should allow clinicians to achieve therapeutic effects comparable to that achieved with single agents, but at substantially lower doses [(239)].

A huge literature is available on the modulation of apoptosis by putative cancer chemopreventive agents, either dietary principles, vitamins, or pharmacological agents [(240)].

## Vitamin A

Vitamin A refers to a group of compounds called retinoids which play an important role in vision, bone growth, reproduction, cell division and cell differentiation [(241)]. Retinoids are active as retinal, retinoic acid and retinol. Retinal is actively involved in supporting vision, retinoic acid regulates cell differentiation, growth and embryonic development and retinol is responsible for transport and storage [(242)].

### Dietary sources of vitamin A

Two forms of vitamin A are found in plants and animals, provitamin A and preformed vitamin A, respectively. Vitamin A found in fruits and green leafy vegetables is called provitamin A carotenoid which is converted into retinol once ingested. The most common provitamin A carotenoids found in foods include beta- carotene, alpha-carotene and beta-cryptoxanthin. Among these, beta-carotene is the most efficiently converted into retinol.Vitamin A found in foods derived from animal sources is called preformed vitamin A and is absorbed as retinol. Liver, whole milk, and select fortified foods are good sources. Interestingly, non-fat milk lacks adequate vitamin A and therefore non-fat milk is fortified with vitamin A. Breakfast cereals are also fortified with vitamin A. Preformed vitamin A is the form supplied by most supplements [(243)], Fig (8).

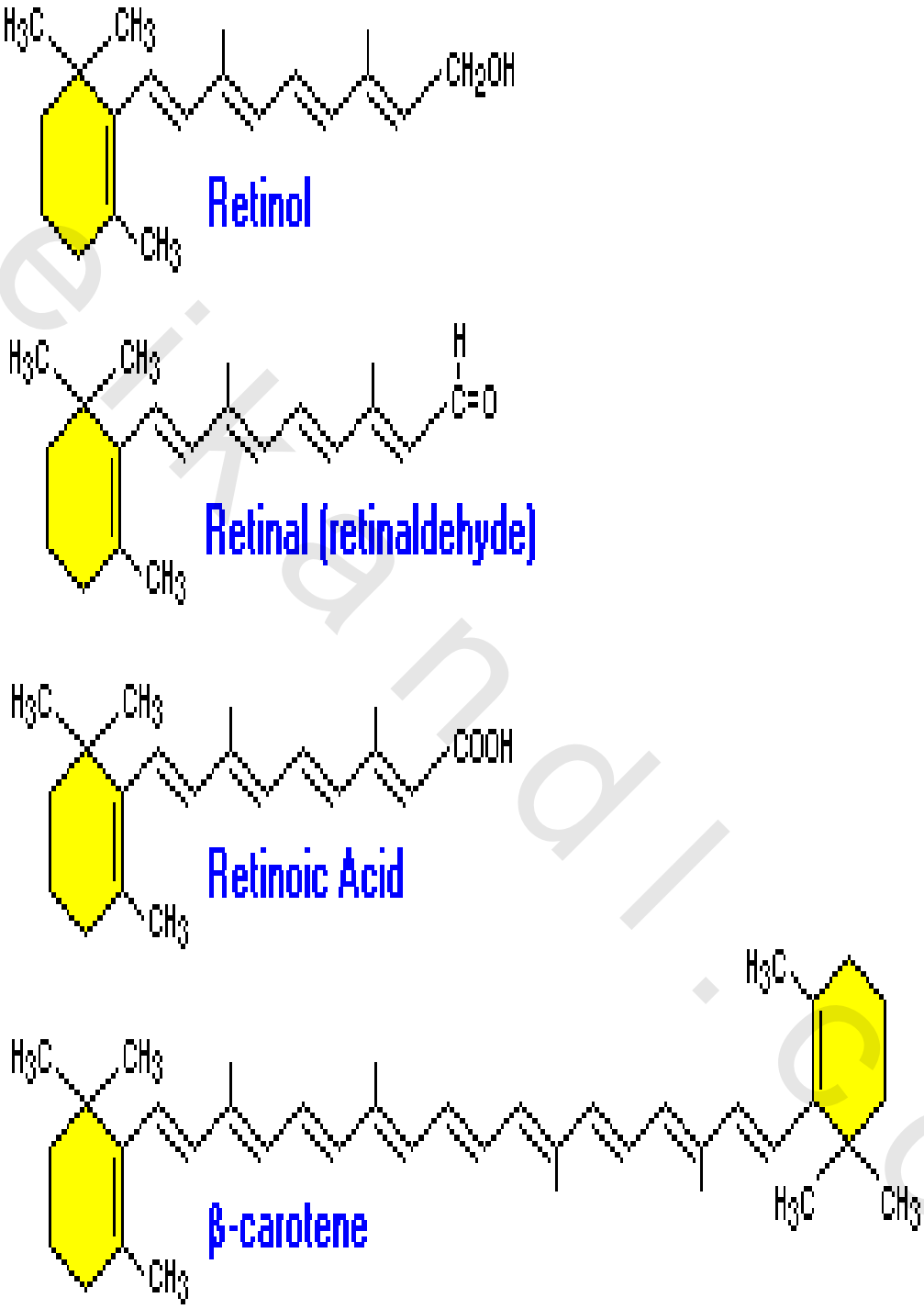



**Fig (8)**: Different forms of vitamin A.

**Metabolism and signaling of vitamin A, Fig (9)**

Vitamin A metabolism and signaling in vertebrates are tightly controlled processes involving multiple levels of regulation, Fig (9). Ingested vitamin A is transported to target tissues in a complex with retinol binding protein (RBP) and transthyretin (TTR) [(244)], where cellular uptake is mediated by the transmembrane receptor stimulated by retinoic acid gene 6 (*STRA6*) [(245)]. Once inside the cell, vitamin A is converted to retinoic acid (RA) in two separate oxidation steps. Vitamin A is first reversibly oxidized into retinaldehyde by alcohol dehydrogenase (ADH) or short-chain dehydrogenase/reductase (SDR) enzymes, such as RDH10 [(246)]. Retinaldehyde is subsequently irreversibly oxidized into RA by retinaldehyde dehydrogenase (RALDH) enzymes, with RALDH2 being the main RA synthesizing enzyme during early embryogenesis [(247)].

Elimination of RA is primarily catalyzed by members of the cytochrome p450 family [(248)]. Intracellular retinoid metabolism is further influenced by cellular retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP) [(249)]. It has been suggested that CRBP acts as a chaperone for vitamin A and may determine the cellular levels of vitamin A accumulation and esterification [(250)], CRABP might facilitate the translocation of RA from the cytoplasm to the nucleus thus acting as a coregulator of RA signaling [(251)].

Retinoids are fat-soluble, animal-derived compounds that include retinol, retinal, and retinoic acids. Ingested retinoids are solubilized and esterified into retinyl esters (RE) in the intestine, circulated as both bound RE and unbound retinol, and stored in the liver as retinol. While the serum retinol concentration is homeostatically maintained, hepatic stores accumulate indefinitely with increased ingestion. Conversely, pro-vitamin A carotenoids are water-soluble, plant-derived compounds that include carotene, lutein, and xanthophylls, which are less bioavailable than retinoids and amounts ingested in excess of needs are not stored but are excreted [(252)].

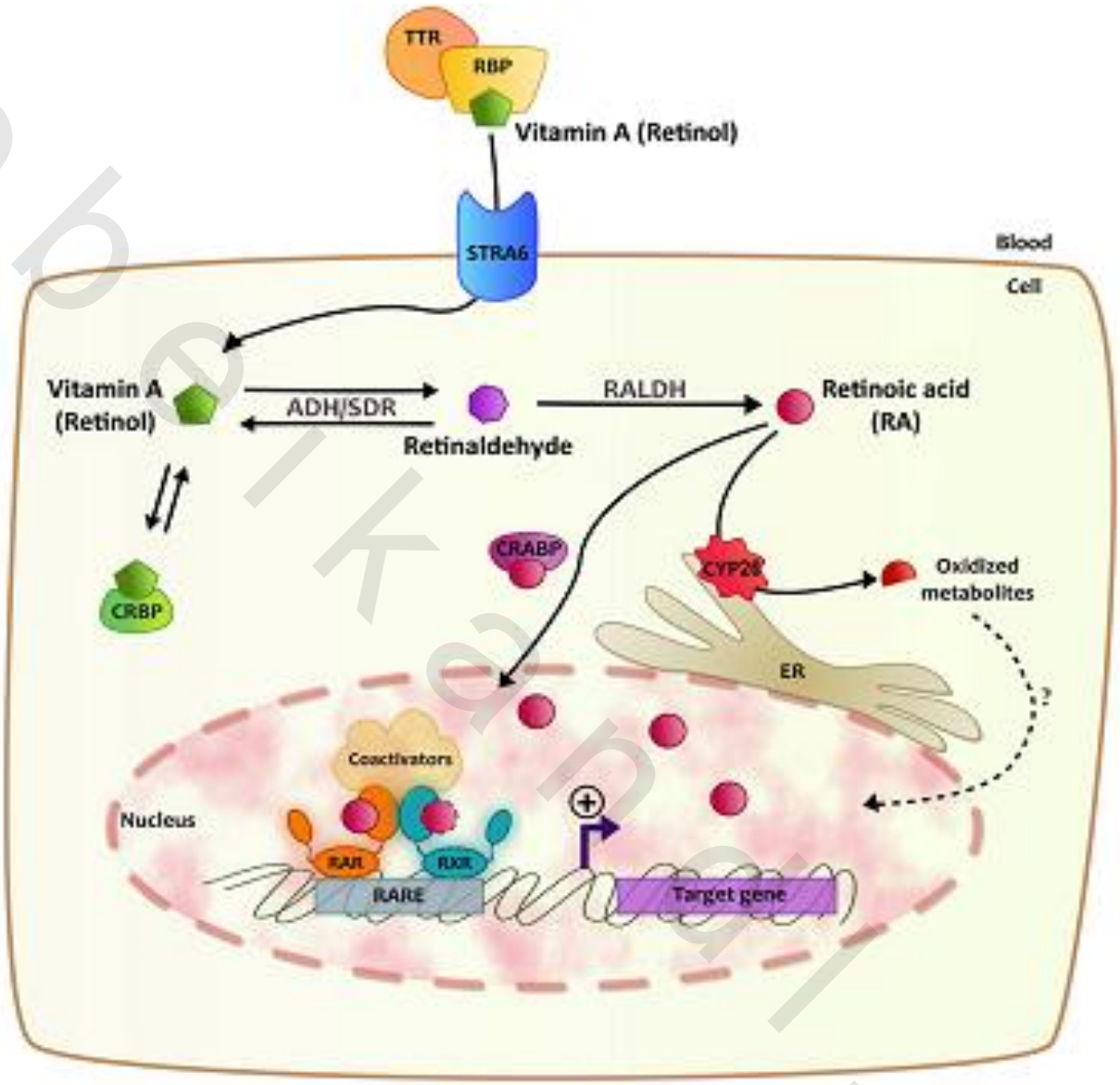



**Fig (9)**: Overview of vitamin A metabolism and signaling. Conversion of vitamin A to its major active metabolite, retinoic acid, and activation of retinoid-dependent signaling. ADH, alcohol dehydrogenase; CRABP, cellular retinoic acid binding protein; CRBP, cellular retinol binding protein; CYP26, cytochrome P450 family 26; ER, endoplasmic reticulum; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RARE, retinoic acid response element; RBP, retinol binding protein; RXR, retinoid X receptor; SDR, short chain dehydrogenase/reductase; STRA6, stimulated by retinoic acid gene 6; TTR, transthyretin.

**Plasma levels of vitamin A**

The plasma concentration of retinol increases with age and in adults is about 2 µmol/L. There is also about 0.2 µmol/L of retinyl esters in chylomicra remnants and in low-density lipoproteins whereas RA is complexed with albumin. Lipoprotein lipase hydrolyses triglycerides present in chylomicra to convert them to chylomicra remnants, containing the retinyl esters, which are finally removed from the circulation mainly by the liver cells which present specific receptors. Other forms of Vit A are found in the plasma for approximately 2–20 nmol/L: 13-cis RA, at-RA, etc. In human plasma, where it is also bound to serum albumin, at-RA is usually at a concentration 150-fold lower than that of retinol [(253)].

**Vitamin A functions**

Vitamin A is required for the proper functioning of a diverse array of metabolic and physiologic activities. Vision, hematopoiesis, embryonic development, skin cell differentiation, immune system function, and gene transcription all require vitamin A [(254)].

Retinoid acid (all-trans retinoic acid, tretinoin) is the biologically active form of vitamin A that ultimately binds to nuclear receptors and facilitates transcription. Intracellularly, retinoic acid is found in all-trans and in 9-cis configurations. All-trans retinoic acid is the predominant form and is the active ligand that binds to the known retinoid receptors. These receptors are found in 2 similar groups—retinoic acid receptors and retinoid X receptors—and belong to a super-family of nuclear receptors that act as transcription factors, which promote the physiologic effects on DNA transcription. These binding properties and transcriptional actions on the nucleus are responsible for the anti-proliferative and anti-inflammatory effects of the retinoids [(255, 256)].

**Vitamin A and breast cancer**

Vitamin A and its derivatives have been long implicated in development and tumor suppression [(257)]. Retinoids have been implicated in the induction of cell death in many tumor-derived cultured cell systems through retinoid receptors–dependent or retinoid receptors–independent mechanisms [(258)]. Retinoids have been shown to be important in oncogenesis for many tissues, and alterations in vitamin A and retinoid homeostasis are found in many tumors, including leukemia, breast, oral, prostate and carcinoma of the cervix [(259, 260)].

Many longitudinal cohort studies have assessed carotenoid intake and endogenous retinol levels and the risk for developing breast cancer among postmenopausal women from multiple ethnic groups and geographic locations worldwide [242, 261–265]. Among postmenopausal women, select studies have concluded that intake of carotenoids and retinol levels are not associated with breast cancer risk [242, 261-263, 266]. When individual carotenoids were evaluated, however, lycopene was associated with a reduction in breast cancer risk among postmenopausal women [261,266, 267].

Retinoic acids (RAs), the natural and synthetic derivatives of vitamin A, are able to induce the proliferation arrest, differentiation, and apoptosis of cancer cells [268]. Studies *in vitro* and *in vivo* have shown that RAs might be effective in prevention and treatment of breast cancer [269, 270].

## Vitamin E

Vitamin E is a lipid soluble group of compounds with similar biological activity to RRR-α-tocopherol. This includes the stereoisomers α-tocopherol, ß- tocopherol, γ-tocopherol, and δ-tocopherol, along with the 4 tocotrienols—α, ß, γ, and δ-tocotrienol, Fig (10), [271].

### Dietary Sources of vitamin E

Vitamin E is a fat-soluble vitamin that can be obtained through dietary intake of nuts, seeds, vegetable oils, green leafy vegetables and fortified cereals or as a supplement. While Vitamin E exists in several chemical forms, it is the alpha-tocopherol form that is preferentially found in human plasma and the most widely studied in clinical trials. Some of the pharmacologic properties of Vitamin E include inhibition of oxidation, inflammation and protein kinase C. Vitamin E also increases the release of prostacyclin which promotes blood vessel dilation and reduces platelet aggregation [241].

The tocopherols are exclusively synthesized in photosynthetic organisms including higher plants; significant amounts are found in all green tissues but predominantly occur in seeds. Plant derived oils represent the major sources of vitamin E in the human diet, and because these oils contain the four tocopherols in different relative amounts, the overall intake of each vitamin E analogue depends in large on the dietary oil preferences in different countries [272]. The tocotrienols

are the major vitamin E components of palm oil, significant amounts are also found in barely, oat and rice bran [273].

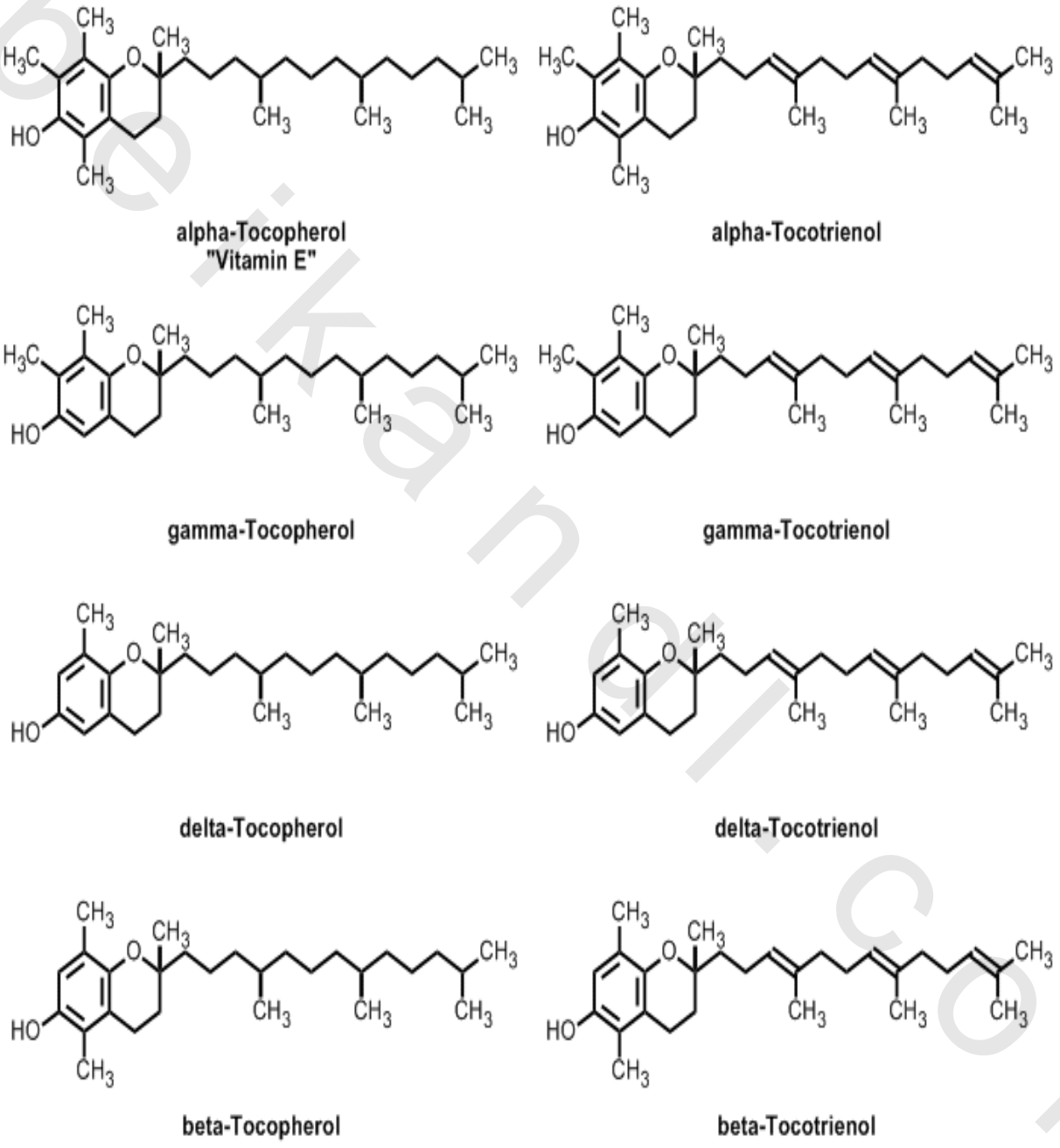



**Fig (10)**: Different forms of vitamin E.

**Plasma α-tocopherol level**

The normal average plasma concentration of α-tocopherol is about 23.2 μM, a plasma level below 11.6 μM is regarded as deficient [(274)]. In human plasma of unsupplemented individuals, average α-tocopherol concentrations (22– 28 μM) are about 10 and 100 times higher than that of γ-tocopherol (2.5 μM) and of δ-tocopherol (0.3 μM) concentrations, respectively [(275)].

**Absorption, transport and metabolism of vitamin E**

Vitamin E is fat-soluble and it is absorbed in the same manner as fat. Specifically unique tiny spheres with a water-loving (hydrophilic) outer layer called micelles engulf the vitamin E and help ferry it across the gut. Chylomicrons, produced by the small intestine, carry the micelles into the lymph, the milky fluid containing white blood cells, proteins, and fats. In the lymph the enzymes lipoprotein lipases break down the majority of chylomicrons to produce chylomicron remnants, which go into the blood. The majority of the chylomicron remnants reach the liver, which strips away the vitamin E from the remnants and puts it into the freshly produced very low-density lipoproteins (VLDL). VLDL is broken down by lipoprotein lipases to produce the low-density lipoproteins (LDL, bad cholesterol.) In our blood LDL is the largest carrier of vitamin E. LDL freely exchanges vitamin E with high-density lipoproteins HDL and LDL seem to deliver vitamin E to our tissues [(276)].

Our blood and tissue contains much more alpha-tocopherol than any of the other tocopherols and tocotrienols. At the heart of this mechanism is a special protein, called the alpha-tocopherol transfer protein. It recognizes the alpha-tocopherol and preferentially puts more of it in the blood [(277)].

**Cellular effects of vitamin E**

In humans, some of the symptoms of vitamin E deficiency, such as increased lipofuscinosis most likely originate from the lack of scavenging ROS in the lipid phase [(278)], whereas others symptoms such as the dying-back sensory axonopathy may also be explainable by other ways, e.g. by modulating gene expression in the brain [(279-281)] and by acting as a survival factor for specific neuronal cells [(282)].

Vitamin E could serve a dual function; primarily, as an essential cofactor acting as a protein-bound molecule in redox-dependent or independent manners, and secondary, when all the other redox active molecules are exhausted or when excess vitamin E is supplemented, by preventing the oxidation of lipids and the propagation of their damaging effects, depending on continuous recycling by L-ascorbic acid. Thus, the continuous removal of oxidized lipids by the vitamin E/vitamin C system may prevent their accumulation in the membrane and consequent rapid depletion of the essential cofactor ‘‘vitamin E’’ [283].

Currently, the molecular and cellular effects of vitamin E have been explained either by acting as a mere antioxidant preventing damage to membranes or proteins and regulating their activity by specifically scavenging ROS and NOS [284], or by interacting and regulating specific enzymes and transcription factors and influencing cellular structures such as membranes and lipid domains [285, 286].

**Extracellular effects of vitamin E**

Vitamin E can also affect extracellular events, e.g. by influencing the extracellular matrix which is centrally involved in the maintenance of an intact vascular wall as well as in the repair of atherosclerotic lesions during disease development [287]. Vitamins E and C modulate the cells present within the connective tissue thus influencing the production and assembly of extracellular proteins and in particular the extracellular matrix of the vascular system [287]. Many possible beneficial effects of vitamin E supplementation, that may go beyond its essential function, were studied with many diseases; in particular with atherosclerosis [288], and other cardiovascular diseases [289, 290], with certain types of cancer [291, 292], with fibrotic disease [293], with a number of neurodegenerative diseases such as Alzheimer’s disease [294], Parkinson’s disease [295], or ataxia with vitamin E deficiency (AVED) [296].

**Vitamin E and cancer**

Cancer chemoprevention is presently acceptable as a strategy for the reduction of cancer occurrence not only in high risk groups but also in the general public. In addition to the search for the identification of chemopreventive agents, the development of effective therapies for cancer is also under thorough investigation. Nevertheless, the search for a single compound to treat all types

of cancer has been unsuccessful to date. Current chemotherapeutic treatments are usually not completely selective for carcinogenic cells with consequent cytotoxic effects on normal cells and therefore decreased quality of life for patients with cancer. Interestingly, recent evidence in the literature suggests that more effective treatments of cancer could be possibly achieved if chemotherapeutic drugs were used with other adjuvant agents with known antitumorigenic activities [297].

During the past decade much evidence has accumulated demonstrating the anticancer activity of specific forms of vitamin E [298, 299]. In the last few years, there has been an explosion of research activity surrounding the potential anti-cancer effects of different vitamin E vitamers. These studies suggest that not only α-tocopherol, but also α-tocopherol and both δ- and γ-tocotrienols might have potent anticancer activities, possibly mediated via anti-angiogenic, anti-proliferative, and pro-apoptotic effects [300-302]. These apparent anti-cancer effects appear to be mediated independently of any antioxidant properties [303, 304].

The antitumor activity of tocotrienols may not be associated with their antioxidant activity only. Based on the recent findings, the anticancer effects can be addressed through at least four mechanisms: antiangiogenesis, antiproliferation, induction of apoptosis and improving immunological functions [305].