

Mechanisms adopted by cancer cells to escape apoptosis—A review

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Abstract: Inactivation of apoptosis is the prime phenomenon in cancer development and cancer treatments. Mutations in the apoptotic pathway not only exert resistance to apoptosis and provide a survival advantage to cancer cells but also confer resistance to cancer therapies. Escaping apoptosis is the “hallmark” of cancer cells. Cancer cells can withstand many apoptotic stimuli, such as DNA damage, unfavorable environments, and cytotoxic therapies. Substantial research has been carried out and is in good progress on the various mechanisms adopted by cancer cells to evade apoptosis. This article reviews the apoptosis escape mechanisms by cancer cells, viz. apoptotic gene alterations (in few essential and accessory apoptotic genes), post-translational modifications (phosphorylation and ubiquitination of apoptotic proteins), metabolic alterations, mitochondrial alterations, immunity escape, epigenetics, cancer cell dormancy, cancer clonal theory, and reversibility of apoptosis. The review reveals that there is a wide scope for further research to address the various challenges in realizing successful cancer therapies that involve reversing the apoptotic resistance and/or inducing apoptosis in tumor cells.

Introduction

Cancer is one of the leading causes of death. As research in the therapeutic approach of cancer is developing, cancer is also developing its resistance towards various therapeutic agents. The most prevalent factor providing resistance to cancer cells is the escape from the default cell death program—‘apoptosis’. In order to survive, cancer cells override many barriers that would cause apoptosis. Apoptosis is a highly regulated cell death process that occurs in multicellular organisms. It plays important role in the organism’s life from embryogenesis to aging, and in many diseases, such as cancer, neurodegenerative diseases, ischemic injury, AIDS (Acquired Immunodeficiency Syndrome), and autoimmune diseases. It is a homeostatic mechanism where organisms eliminate unnecessary cells from the body in the course of development, mop out infected or damaged cells from the system, and maintain the level by replacing these cells with the new ones. In response to the apoptotic stimuli, a complex cascade of events causes changes such as chromatin condensation, membrane blebbing, cell shrinkage, nuclear fragmentation, and DNA fragmentation, eventually leading to the cell demise without eliciting an immune response (Sjöström and

Bergh, 2001). The process is carried out by cysteine aspartate proteases recognized as caspases. Many human diseases occur due to either the death of cells that should live or the presence of cells that should die. Hence, realizing the fact that apoptosis has the ability to restrict abnormal growth of tissue, the research interest on apoptosis regulation has suddenly emerged. By extensive research on diseases showing a characteristic decrease in apoptotic rate and on how the diseased cells escape apoptosis, we may be able to use these escape mechanisms to treat the diseases characterized by an increased rate of apoptosis.

The mechanisms of apoptosis evasion by cancer cells are of central importance in drug development as many cancer therapies intend to initiate cell death. Many researchers have elaborated the diverse aspects of cancer and apoptosis, such as apoptosis-inducing drug resistance due to apoptotic gene modifications and targeting these modifications to develop new therapies (Lowe and Lin, 2000; Schmitt, 2003; Mashima and Tsuruo, 2005; Fulda, 2010; Wong, 2011; Kaleigh and Kurokawa, 2013; Dasgupta *et al.*, 2017; Aleksakhina *et al.*, 2019; Jan and Chaudhry, 2019), cancer cell interaction with immune system and immune escape mechanisms (Messerschmidt *et al.*, 2017; Mohme *et al.*, 2017; Rodriguez, 2017; Leone *et al.*, 2018; Steven and Seliger, 2018), cancer cell and their microenvironment (Gao *et al.*, 2017; Sun *et al.*, 2019), epigenetics (Yan *et al.*, 2017; Hervouet *et al.*, 2013), and anti-tumor drugs (Yan *et al.*, 2017; Rodriguez *et al.*, 2013;

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Cesari *et al.*, 2014; Leone *et al.*, 2018). However, the present article provides a comprehensive review of the various apoptosis-escape mechanisms reported so far, such as genetic alterations, post-translational modifications, metabolic alterations, mitochondrial alterations, immune system escape, epigenetics, cancer stem cells (CSCs), cancer cell dormancy, cancer clonal theory, circulating and disseminated tumor cells (CTCs and DTCs), and reversibility of apoptosis. After sequentially covering these sub-topics, the article highlights the challenges and future research directions to realize these mechanisms in cancer therapies and ends with concluding remarks.

Pathways to initiate apoptosis

The two most common pathways to initiate apoptosis are denoted as intrinsic and extrinsic (Sjöström and Bergh, 2001; Kessel, 2015). The intrinsic pathway can be termed as auto-activation as the cell itself senses the stimuli and undergoes apoptosis, while the extrinsic pathway is paracrine-activation, where the cell receives signals from neighboring cells and extracellular space; both pathways work by the activation of caspases (Elmore, 2007).

In the extrinsic pathway or death receptor pathway, extracellular ligands activate the responsible receptors that are located in the cell membrane. According to Kaleigh and Kurokawa (2013), the engagement of extracellular ligands such as FAS (FS-7-Associated Surface antigen) and TNF (Tumor Necrosis Factor) with cell surface receptor leads to the formation of DISC (Death Inducing Signal Complex) and eventual activation of initiator caspase-8 and caspase-10. Caspase-8 has a dominant function in the extrinsic pathway. When the death ligand binds to its cognate receptor, the cell recruits receptor-specific adaptor proteins like FADD (Fas-Associated Death Domain), which forms DISC that further activates caspase-8. The activated caspase-8 either directly catalyzes the activation of execution caspase or acts on its substrate BID (BH-3 Interacting Domain death agonist) to form tBID (truncated BID) that leads to the mitochondrial release of cyt c (cytochrome complex). Studies have suggested the inexplicable dual role of caspase-8 as pro-apoptosis and pro-survival (Salvesen and Walsh, 2014).

The intrinsic pathway (also known as the mitochondrial pathway) is activated by diverse stimuli (extra- and intracellular) like oxidative stress, irradiation, and cytotoxic drug treatment. The pro-apoptotic BCL-2 (B-Cell Lymphoma 2) family members like BIM (Bcl-2 Interacting Mediator of cell death), BID (BH-3 Interacting Domain death agonist), BAD (Bcl-2 Associated Agonist of cell death), BAX (Bcl-2 Associated Protein X), and BAK (Bcl-2 Homologous Antagonist Killer) promote MOMP (Mitochondrial outer membrane permeabilization) due to which pro-apoptotic proteins (such as cyt c) are released from mitochondrial intermembrane into the cytoplasm, where cyt c forms "Apoptosome" by binding to adaptor protein APAF-1 (apoptotic protease activating factor) and then it binds to procaspase-9 (Schafer and Kornbluth, 2006). Apoptosome is a caspase-9-activating complex, which activates initiator caspase-9. The activated caspase-9 sequentially activates caspase-3 that initiates the proteolytic reactions. Along with cyt c, mitochondria release other

polypeptides, including AIF (Apoptosis-Inducing Factor), Endonuclease G, SMAC (Second Mitochondrial Activator of Caspases) also known as DIABLO, and Serine proteases Omi (also known as HtrA2), from the intermembrane space (Elmore, 2007). AIF and endonuclease G cause DNA damage and condensation. And SMAC binds to XIAP (X-linked Inhibitor of Apoptosis Protein), which inhibits several caspases (caspase -3, -7, -9); this binding neutralizes XIAP and facilitates cyt c-induced caspase activation (Chipuk *et al.*, 2006).

The granzyme pathway is another pathway to initiate apoptosis, where CTLs (Cytotoxic T Lymphocytes) kill the damaged/ infected cells by secreting perforin (Trapani and Smyth, 2002). Perforin is a transmembrane pore-forming molecule that forms a pore in the target cell membrane and then releases granules (containing Serine proteases, granzyme A & B) through these pores into the target cell cytoplasm. Other pathways inducing apoptosis might also exist, but the intrinsic and the extrinsic pathways have been studied widely and demonstrated in detail.

The execution phase/pathway is where the abovementioned pathways converge to the final phase that starts by activation of execution caspases (Fig. 1). The execution of several caspases (caspase -3, -6, -7) activates cytoplasmic endonuclease and protease, which degrades nuclear material and proteins, respectively, ultimately leading to morphological and biochemical changes (Slee and Adrain, 2001). Among the execution caspases, caspase-3 is the most frequently activated during apoptosis. It is activated in apoptotic cells by extrinsic, intrinsic, and granzyme pathways where the activation is catalyzed by caspase-8, caspase-9, and granzyme B, respectively. As the execution involves the destruction of cellular structures (like chromatin condensation, DNA fragmentation, cleavage of many key cellular proteins, and formation of apoptotic bodies), caspase-3 is also termed as executioner caspase. Studies reported that caspase-3 also has functions before the cell commits to death (Porter and Jänicke, 1999).

How cancer cells escape apoptosis

In cancer, some of the body's cells divide irregularly without stopping and spread to other tissues in the body. Cancer occurs due to various genetic causes, and it is a complex process involving multiple steps in its development, such as mutation, tumor formation, and metastasis. Tissue homeostasis is affected not only by over-proliferation of the cells but also by decreased removal of cells, i.e., being a "defective cell," cancer cells also need to undergo apoptosis. But during tumor development, these cells acquire few genetic alterations and adopt few mechanisms that cause evasion of apoptosis: one of the hallmarks of cancer. For decades, to find out the mechanisms in cancer cells to escape apoptosis is a topic of high interest to researchers.

Genetic alterations

The outcome of each apoptotic phase is regulated by the various factors involved in apoptosis, genes, and their interactive networks. These apoptotic regulatory genes are often dysregulated in cancer cells. These genomic

abnormalities provide proliferative and survival advantages to the cancer cells.

One of the types of alterations cancer cells use to evade apoptosis is a shift in the balance between pro- and anti-apoptotic gene expression programs. Most tumor cells evade apoptosis by either increasing the expression of pro-survival genes (anti-apoptotic) or decreasing the expression of pro-apoptotic genes. These modulations in gene expressions can be achieved by transcriptional and translational alterations such as gene-deletion, -silencing, -copy number amplification, and transcription factors' activation or inactivation, thereby affecting the expression of apoptotic regulators (Kumar and Cakouros, 2004). Also, mutations in genes that regulate apoptosis are commonly observed in cancer cells; this phenomenon eliminates the pro-apoptotic proteins and/or amplifies anti-apoptotic proteins, a key step in the progression to cancer. Somatic mutations and germline mutations of apoptosis-related genes were reported in human cancers (Pai *et al.*, 1998; Park *et al.*, 2001; Lee *et al.*, 2004; Ghavami *et al.*, 2009; Timofeev *et al.*, 2019). Even the loss of anti-oncogenes (or tumor-suppressor gene) has been observed regulating the cell cycle by controlling growth and proliferation (Levine and Puzio-Ku, 2010). Mutation or inactivation of these genes results in reduction or loss of their function (leading to abnormal cell growth), which is believed to be a key factor in the development of several tumors. In human cancers, the mutation of the anticancer gene family member loses its original function. Tumor suppressor genes are inactivated by point mutations or deletion in both alleles of the gene (Wang *et al.*, 2018). When tumor suppressor genes are inactivated, cell cycle control is lost, and the cell displays uncontrolled growth and division. The “loss of

function” of multiple tumor suppressor genes is considered to be the main reason causing malignancy (Lam and Schmidt, 2012). Tumors also display resistance to receptor-induced cell death (Mohammad *et al.*, 2015).

Genetic studies have identified many genes that function in apoptosis (Hoepfner *et al.*, 2001), and few of the essential and secondary apoptotic genes altered in cancer cells are discussed in the following sub-sections.

Essential genes

BCL-2 genes

BCL-2 (B-Cell Lymphoma-2) gene was originally found in B-cell follicular lymphoma; the pro-apoptotic and anti-apoptotic genes are members of this family (Fig. 2). In normal cells, the sensitivity of cells to apoptotic stimuli depends on the balance between pro-apoptotic and anti-apoptotic BCL-2 proteins. It has been reported that genetic inactivation of a BH3-only (BCL-2 Homology 3-only) protein leads to resistance to certain pro-apoptotic stimuli and also accelerates tumor formation in mice (Faqar-Uz-Zaman *et al.*, 2018).

Over-expression of anti-apoptotic *BCL-2* was observed in cancer cells, e.g., amplification of *BCL-XL* in lung cancer and giant-cell tumor of bone (Adams and Cory, 2007); and amplification of anti-apoptotic *MCL-1* in breast and lung cancers (Faqar-Uz-Zaman *et al.*, 2018; Soderquist *et al.*, 2018). In more than 80% of the bladder cancer tissues, anti-apoptotic BCL-2 protein was found positive, and its expression was correlated with the stage and grade of the tumor (Konstantinidou *et al.*, 2002). Several cancer types such as lung-, breast-, prostate-, gastric-, renal-, pancreatic-, colorectal- and hepatocellular- cancer showed overexpression of members of *BCL-2* family genes (Yip and Reed, 2008;

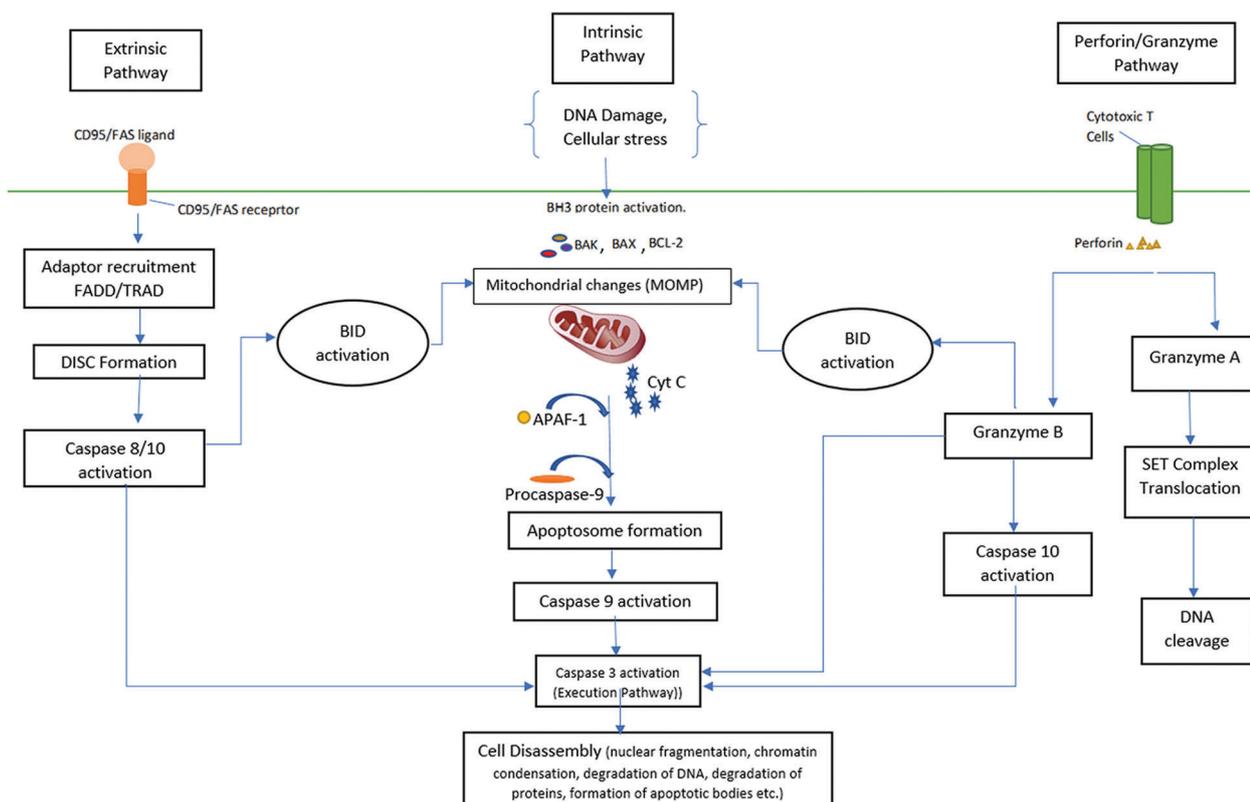


FIGURE 1. An overview of apoptotic pathway.

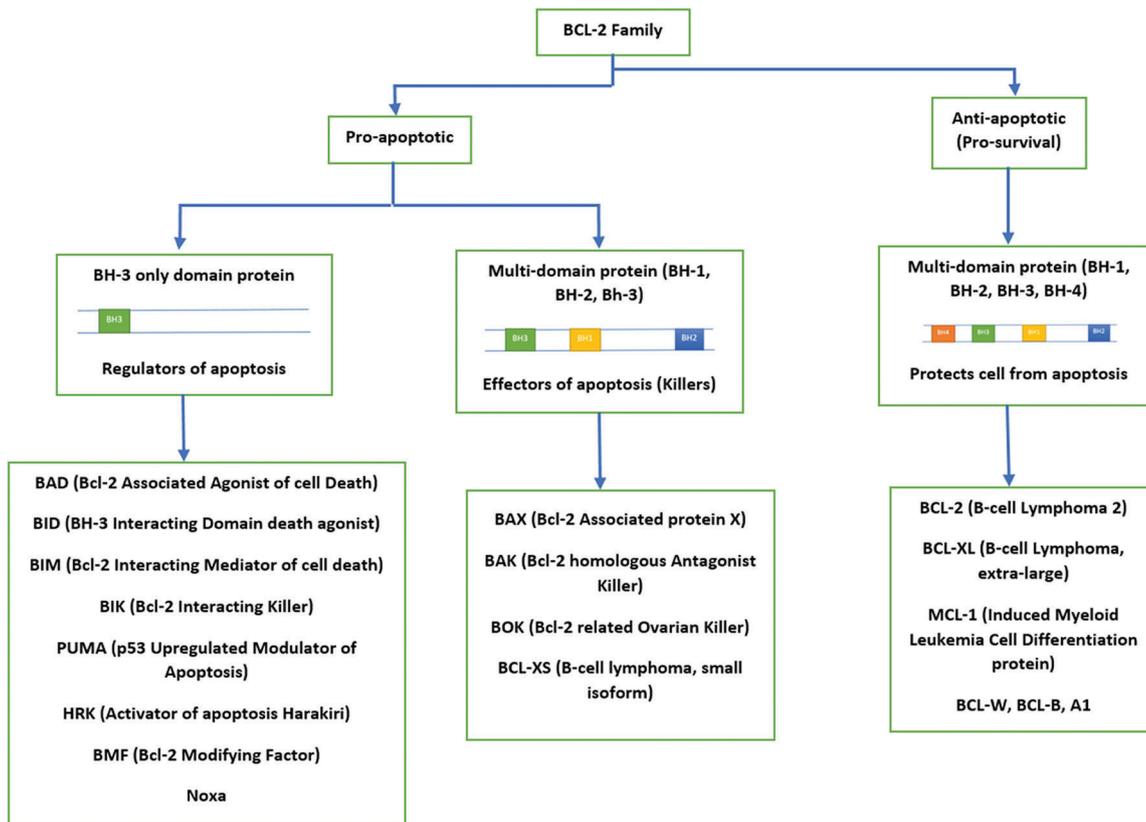


FIGURE 2. Family of BCL-2 genes.

Lessene et al., 2008). A study on transgenic mice reported collaboration of *BCL-2* and/or *BCL-XL* expression with oncogene (Wang et al., 2003). Alternatively, the transcription factors like CREB (cAMP response element-binding protein) and STAT3 (signal transducer and activator of transcription 3), which activate genes in response to pro-survival stimuli, can increase the expression of *MCL-1* and *BCL-2* (Wang et al., 1999; Real et al., 2002). Hence, activation of CREB or STAT3 could promote the expression of anti-apoptotic genes in cancer cells.

Gene coding for pro-apoptotic protein BAX was found mutated in human hematological malignancies (Liu et al., 2013). In some lymphomas and leukemia, due to *BAX* frame-shift mutations, the *BAX* translation is terminated prematurely, due to which BAX loses its function, ultimately leading to apoptosis resistance (Brimmell et al., 1998). Deletion polymorphism of one *BCL-2* family member, *BIM*, was reported in certain human populations and is significantly associated with innate resistance to TKI (Tyrosine Kinase Inhibitor) therapies (Kaleigh and Kurokawa, 2013). *BIM* transcription is induced in response to the withdrawal of growth factors (or other apoptotic stimuli). FOXO is one of the transcription factors involved in *BIM* expression (Jain et al., 2013); the FOXO activity is suppressed by pro-survival kinases, AKT, and ERK. Thus, cancer cells with high levels of AKT/ERK show suppressed *BIM* expression (Costa et al., 2007; Hübner et al., 2008).

Beroukhim et al. (2010) found two *BCL-2* family genes deleted in cancer; a pro-apoptotic BH-3-only family gene *PUMA* (p53 Up-regulated Modulator of Apoptosis) and a pro-apoptotic multi-BH-domain *BOK* (Bcl-2 Related

Ovarian Killer). *PUMA* is found to be most frequently deleted in cancer. *PUMA* is a crucial mediator of p53-dependent and p53-independent apoptosis; it is expressed in response to apoptotic stimuli and regulated by p53. On receiving death signals, *PUMA* binds to anti-apoptotic *BCL-2* family member in mitochondria and relieves the inhibition on BAX and/or BAK, which leads to mitochondrial dysfunction and caspase activation (which carries out apoptosis). *PUMA* inhibition causes apoptotic deficiency and increases the risk of cancer development. According to a few reports, *PUMA* can directly activate BAX/BAK to induce mitochondrial function (Bean, 2014). Cancer cells with compromised functions of *PUMA* were observed in many studies. For instance, the reduction in *PUMA* expression in malignant cutaneous melanoma was reported by Karst et al. (2005). Later, Adams and Cory (2007) stated that “frequent overexpression of anti-apoptotic *BCL-2* family proteins and other anti-apoptotic oncoproteins in tumor antagonizes *PUMA*-induced apoptosis”. Additionally, mutant p53 in human tumors repudiates induction of *PUMA* by irradiation and by chemotherapeutic drugs (Yu and Zhang, 2008). Garrison et al. (2008) also found lower expression of *PUMA* in approximately 40% of human Burkitt’s lymphomas. Loss of *PUMA* in the hypoxia-induced tumor model causes chromosomal instability and promotes tumorigenesis (Nelson et al., 2004). Further research is needed to prove the role of *PUMA* in tumorigenesis because few studies suggest that *PUMA* is not directly inactivated in cancers, rather its loss is the outcome of alterations in other genes (Yoo et al., 2007).

TP53(Tumor protein 53) gene

The *TP53* gene codes for the transcriptional factor p53, which plays important roles in apoptosis. It is observed that *TP53* is the most frequently mutated or inactivated tumor suppressor gene in cancer. More than half of human tumors contain *TP53* mutations (Vogelstein and Kinzler, 2004; Oren and Rotter, 2010). In normal cells, in response to stress, stabilized p53 combines with other p53 subunits and functions as a critical transcription factor that modulates the expression of an array of genes known to trigger apoptosis. p53 promotes activation of *GADD45* (*Growth Arrest and DNA Damage 45*) and *P53R2* (*p53 controlled Ribonucleotide reductase 2*) genes, which repairs damaged DNA, due to which mutation (this DNA damage) is not accumulated and is not passed to the daughter cells. However, when DNA changes are irreparable, p53 activates the genes involved in apoptosis, such as *PUMA*, *BAX*, *FAS*, *PIG3* (*p53 Inducible Gene 3*), and *Killer/DR5* (*Death Receptor 5*), which leads to apoptotic death of the cells with damaged DNA.

The p53 protein regulates glycolysis, PPP (pentose phosphate pathway), mitochondrial oxidative phosphorylation, lipids and nucleotides metabolism, and the cell response to oxidative stress (Perri *et al.*, 2016). Tumor cells depend mainly on glycolysis for energy (referred to as the Warburg effect). p53 down-regulates glycolysis by inducing genes *TIGAR* (*TP53 Induced Glycolysis and Apoptosis Regulator*), which inhibits glycolysis, and by inducing genes *SCO2* (synthesis of cytochrome oxidase 2) and *GLS2* (mitochondrial glutaminase 2), which promotes oxidative phosphorylation (Jiang *et al.*, 2011). Moreover, glycolysis is down-regulated by inhibiting the expression of *GLUT1* (Glucose Transporter 1) and *GLUT4* (Glucose Transporter 4) due to which glucose uptake is blocked (Jiang *et al.*, 2011). Other than glycolysis, PPP (Pentose Phosphate Pathway) is an alternative pathway for tumor cells; p53 inhibits PPP as well, by reducing the activity of enzyme *G6PD* (glucose-6-phosphate dehydrogenase) (Perri *et al.*, 2016). p53 also arrests fatty acids biosynthesis by acting on *FASN* (fatty acids synthase) and *ACLY* (ATP citrate lyase) and inhibits the formation of the membrane in tumor cells (Freed-Pastor *et al.*, 2012).

In cancer cells, a mutation in *TP53* leads to the formation of mutant p53 protein. Liu *et al.* (2014) stated that “most p53 mutations in human cancer are missense mutations, which result in the production of full length mutant p53 proteins”. 10–15% of *TP53* mutations (termed as ‘disruptive mutations’) produce inactive proteins, and the remaining 85–90% of mutations form functioning proteins. Thus, mutations affect the ordinary function of p53 as a transcriptional factor, which imparts survival advantage to cancer cells and eventual down-regulation of apoptosis. Furthermore, several studies have demonstrated that mutant p53 is not only unable to perform tumor suppressor function, but also gains new oncogenic functions (termed as “gain-of-function”) (Oren and Rotter, 2010; Liu *et al.*, 2014). A recent study on myelodysplastic syndromes reported the role of *TP53* dysfunction in cancer outcomes and suggested its use in diagnosis (Bernard *et al.*, 2020).

Caspase genes

Caspase genes encode protease enzymes called caspases. They are named caspase because of their specific cysteine protease

activity, and they have an essential role to play in apoptosis. Based on their pro-apoptotic functions, caspases are divided into two groups: initiators and effectors. The activated first group of initiator (or apical) caspases (-2, -8, -9, -10, and -11) in turn activate the second group of caspases (-3, -6, and -7) (Li and Yuan, 2008). At times, initiator caspases equally act as effector caspases to turn up weak suicide signals (Ghavami *et al.*, 2009). Playing a crucial role in apoptosis, caspases are obliged to be targeted in uncontrolled cell division. P53 transcriptionally regulates the expression of some caspase genes (-2, -7, -8, and -9) (Ghavami *et al.*, 2009); in cancer cells where p53 is mutated, expression of these caspases could be compromised, resulting in apoptotic protection.

According to Soung *et al.* (2005), various cancers showed the presence of mutated caspase-8 (mutations such as missense, in-frame deletion, frame-shift mutations in the coding sequences, mutations in the initiation codon, mutations in the introns, and mutation in the untranslated region), and there was a remarkable decrease in the apoptosis-inducing activity of all mutated caspase-8. For instance, missense mutations resulted in the substitution of amino acids, and frame-shift mutations caused premature terminations of caspase-8 protein synthesis (Ghavami *et al.*, 2009; Li *et al.*, 2014). Caspase-8 mutations were mostly detected in gastric cancers (Soung *et al.*, 2005), though rarely observed in other types of cancer. Sun *et al.* (2007) identified a 6 bp deletion polymorphism (2652 6N del) in the promoter of the *CASP8* (*Caspase 8*) gene, which lowers caspase-8 protein level, which is associated with 25% increased risk of lung-, esophageal-, stomach-, colorectal-, breast- and cervical- cancers. The absence of caspase-8 protein precursor in cancer cells was reported by Hopkins-Donaldson *et al.* (2003). *CASP8* variants were observed in breast cancer (Cox *et al.*, 2007). Yang *et al.* (2008) examined the association of *CASP8* deletion polymorphism with pancreatic cancer. Silent mutations of *CASP9* (*Caspase-9*) are found in gastric- and colorectal- carcinoma (Soung *et al.*, 2006). A case-control study found an association of *CASP9* polymorphism with multiple myeloma (Hosgood III *et al.*, 2008).

Caspase-3 is an effector caspase playing an essential role in the execution phase of cell apoptosis. Soung *et al.* (2004) studied *CASP3* (*Caspase-3*) mutation in carcinomas, myelomas, and lymphomas, suggesting the presence of *CASP3* mutation in human cancer tissues. They reported *CASP3* mutations in several cancers (stomach carcinoma, colon carcinoma, breast carcinoma, non-small cell lung cancer, laryngeal carcinomas, hepatocellular carcinoma, esophagus carcinomas, renal cell carcinomas, urinary bladder carcinomas, medulloblastomas, Wilms’ tumors, non-Hodgkin lymphomas, acute leukemias, and multiple myeloma) and compared seven healthy malignant tissues from the same patients; they found no *CASP3* mutations in normal samples and concluded that *CASP3* mutations arise somatically. They also found that *CASP3* mutations consisted of missense mutations, silent mutations, mutations in the introns, and mutation in the untranslated regions in few cancers (stomach adenocarcinoma, lung cancer, colon cancer, hepatocellular carcinoma, and multiple myeloma).

SCCHN (Squamous Cell Carcinoma of the Head and Neck) is one of the most common cancers in the world (Li *et al.*, 2014), and association of *CASP3* polymorphism with an increased risk of SCCHN has been reported (Chen *et al.*, 2008; John *et al.*, 2015). Caspase-7 is another effector caspase playing a central role in executing apoptosis. Some common cancers (such as SCCHN, Urinary bladder carcinoma, esophageal carcinoma, lung cancer, colon adenocarcinoma, and gastric carcinoma) displayed *CASP7* (Caspase-7) somatic polymorphism (Soung *et al.*, 2003; Yoo *et al.*, 2004). *CASP7* mutations found in this study were non-sense mutations causing premature termination of protein synthesis forming loss-of-function protein. Arg-43 is a highly conserved amino acid in caspase-7; alteration of Arg-43 decreases the protease function of caspase-7 that has a defect in the induction of apoptosis (Soung *et al.*, 2003).

Death Receptor genes

DR5 (Death Receptor 5)- The KILLER/DR5, also known as *TRAILR2* (TNF-related apoptosis-inducing ligand receptor 2), has been identified as a potent inducer of apoptosis. *KILLER/DR5* gene codes for cell surface receptors having a death domain, and over-expressed *KILLER/DR5* gene is an effective apoptotic inducer (Wu *et al.*, 2000). Binding of DR5 to its specific ligand TRAIL (TNF-related apoptosis-inducing ligand) mediates the apoptotic effects of TRAIL by activating caspase cascade (Griffith and Lynch, 1998). According to Takimoto and El-Deiry (2000), the expression of the *KILLER/DR5* gene prevents cancer development and helps to carry out p53-induced apoptosis in cells with wild type p53, but this gene is affected in cells with inhibited p53 function. They also suggested that *KILLER/DR5* alterations have a role in tumorigenesis.

Park *et al.* (2001) showed frequent allelic loss of *KILLER/DR5* in cancers, mostly in gastric cancer. There is accumulating evidence suggesting that the mutation of the primary structure of DR5 might be one of the possible mechanisms that disrupt apoptosis in tumor cells. Cancer cells have escaping mechanisms from DR5-mediated apoptosis such as by imitating the expression of DR5 receptor, by loss of DR5 expression, overexpression of inhibitory proteins like FLICE (FADD-like Interleukin-1beta- Converting Enzyme), and mutation in structural genes of DR5 (Park *et al.*, 2001; Karbasi *et al.*, 2015). By analyzing the genetic alterations of *KILLER/DR5* in a number of gastric cancers, Park *et al.* (2001) found the loss of function of *KILLER/DR5* mutants. Further, they found all the mutants inhibited apoptotic cell death in transfection studies, suggesting that inactivation of *KILLER/DR5* due to mutations might be one of the possible escaping mechanisms against *KILLER/DR5* (extrinsic) mediated apoptosis, and this might contribute to the development of gastric cancers. Pai *et al.* (1998) performed a sequence analysis of *KILLER/DR5* and found mutations localized to the functional cytoplasmic death domain; alterations in this domain resulted in a truncated protein. This *KILLER/DR5* mutation was also present in the germline of the affected patient. Gene coding for DR5 was found on chromosome 8p21-22 (MacFarlane *et al.*, 1997), and several cancers such as gastric cancer (Yustein *et al.*, 1999), lung cancer (Sug

Hyung Lee *et al.*, 1999), head and neck cancer (El-Naggar *et al.*, 1998) showed frequent allelic losses in this chromosome.

FAS gene-FAS codes for FAS receptor (containing death domain), which is also known as CD95 (Cluster of Differentiation 95) or APO-1 (Apoptosis Antigen-1) (Wajant, 2002). In the extrinsic pathway, FAS ligand binds to FAS receptor or TNF ligand binds with TNF receptor, resulting in binding of TRADD (TNF Receptor type 1-Associated Death Domain) with FADD, and RIP (Receptor Interacting Protein) (Kelliher *et al.*, 1998; Wajant, 2002). Subsequently, the FADD-procaspase-8 association leads to the formation of DISC (Wang *et al.*, 2010). Apart from its crucial role in apoptosis, FAS was observed to have non-apoptotic functions such as differentiation or proliferation of the cell and facilitating the pathogenesis of various malignancies (Krammer, 2000). Edathara *et al.* (2016) found that FAS gene polymorphism discouraged the apoptotic activity of its receptor, and this altered FAS receptor was associated with tumorigenesis. Further study by Cavalcante *et al.* (2019) found an association of FAS polymorphisms with ATL (Adult T-cell Leukemia). According to O'Reilly *et al.* (2009), a mutation in *FASL* (FAS ligand) prevents its binding to the receptor and retracts the function of membrane-bound *FASL* and soluble *FASL*. Studies reported that membrane-bound *FASL*-FAS and soluble *FASL*-FAS can enhance the development of cancer (Kaufmann *et al.*, 2012; Waring and Mullbacher, 1999).

APAF-1 gene

APAF-1 is trans-activated by p53 (Steele *et al.*, 1998; Soengas *et al.*, 2001). This gene codes for a cytoplasmic protein APAF-1. APAF-1 acts downstream of p53 to induce apoptosis. It forms one of the important units, "apoptosome", in the apoptosis process, which contains a CARD (Caspase Recruitment Domain). The apoptosome binds to Procaspase-9, cleaves it, and releases caspase-9 (Shakeri *et al.*, 2017). Salvesen and Duckett (2002) pointed out that despite the current understanding of the mitochondrial pathway of apoptosis, many details are yet to be described clearly, such as the molecular mechanism of APAF-1 oligomerization and activation of caspase-9 by APAF-1.

A study on mice reported APAF-1 deficient cells displayed resistance to apoptotic stimuli and showed reduced apoptosis (Yoshida, 2003). Hence, APAF-1 is considered an essential tumor suppressor downstream of p53. A study on drug-resistant tumor cells showed that anti-cancer drugs released cytochrome-c but failed to activate caspase-9; APAF-1 expression was down-regulated in these drug-resistant malignant melanomas, suggesting that impairment in APAF-1 provides proliferative benefit to malignant cells (Soengas *et al.*, 2001). Few other studies also proved the role of APAF-1 in tumorigenicity (Soengas *et al.*, 1999; Paik *et al.*, 2007), while the loss of APAF-1 expression was reported in several types of cancers, such as colorectal cancer (Zlobec *et al.*, 2007), gastric cancer (Wang *et al.*, 2007), bladder cancer (Hinz *et al.*, 2007), and melanoma (Dai *et al.*, 2004).

A study on human neuroblastoma cell lines found that overexpression of *miRNA-3613-3p* decreased transcription of *APAF-1* mRNA, resulting in down-regulation of APAF-1,

which indicates that the key molecule inhibiting apoptosis in BE (2)-C human neuroblastoma cells is APAF-1 (Nowak *et al.*, 2018).

Secondary genes

Proto-oncogene

Proto-oncogenes are highly regulated protein-coding genes regulating cell growth and cell survival signals (Wang *et al.*, 2018). Proto-oncogenes can be altered by chromosomal translocation, mutation, gene amplification, or retroviral insertion. If it gets mutated, it becomes oncogene and contributes to cancerogenesis, i.e., gain-of-function mutation (McDuff and Turner, 2011). Oncogenes abrogate cell cycle checkpoints, thus promote irregular cell proliferation (Lam and Schmidt, 2012).

Proto-oncogene *MYC* codes for multifunctional phosphoprotein that acts as a transcription factor and plays important role in cell cycle progression, apoptosis, and cellular transformation. According to a study, *MYC* transactivates its target genes to deploy its oncogenic effect (Grandori *et al.*, 2005; Hoffman and Liebermann, 2008). Miller *et al.* (2012) found that mediators of metabolism, biosynthesis, and cell cycle progression are genes targeted by *MYC*, and irregularity in *MYC* expression leads to uncontrolled cell growth, uncontrolled division, and metastasis. *MYC* is frequently aberrated in human cancers, and overexpression of *MYC* confers the progression of 40% of tumors (Mohamed, 2017). Various genetic alterations, such as chromosomal translocations, amplifications, point mutations, and epigenetic reprogramming, are involved in the deregulation of *MYC* expression.

Studies found the association of translocations and/or amplification of *MYC* with Burkitt lymphoma, follicular lymphoma, mantle cell lymphoma, hematopoietic and non-hematopoietic tumors (like lung, breast, colon, and prostate cancers), and multiple myeloma; insertional mutagenesis in avian leucosis virus (ALV)-induced hematopoietic tumors were also observed (Mohamed, 2017; Nguyen *et al.*, 2017).

Non-protein coding *microRNAs* (*miRNA*) are also *MYC* target genes; *MYC* represses *miRNA-15A/miRNA16-1* (tumor suppressor) and *miR-34* (Apoptotic regulator) (O'Donnell *et al.*, 2005). This *MYC-miRNA* interaction promotes malignant phenotype (Jackstadt and Hermeking, 2015) (Details are given under “Proto-oncogene”). *MYC* plays an important role in mitochondrial biogenesis by inducing nuclear-encoded mitochondrial genes and by binding to the promoters of genes encoding proteins involved in mitochondrial function. Additionally, the genes involved with mitochondrial biogenesis are among the *MYC* target genes (Gao *et al.*, 2009). The *MYC* oncogene contributes to the genesis of many human malignancies (Wang *et al.*, 2014).

miRNAs in apoptosis

miRNAs are non-coding RNAs made up of approximately 22 nucleotides (Hwang and Mendell, 2006), which were first discovered in *C. elegans* (Bagga *et al.*, 2005). Later on, human genome studies discovered more than 300 *miRNAs* and estimated up to 1000 *miRNA* genes (Hwang and Mendell, 2006). *MicroRNAs* are involved in post-transcriptional gene regulation (Nowak *et al.*, 2018) and

important mediators in critical cellular pathways. *miRNAs* function by base-pairing with their complementary sites within 3'UTR of their target mRNAs (Hwang and Mendell, 2006; Cui and Placzek, 2018). Apart from playing an important role in cancer progression (Slattery *et al.*, 2018), *miRNAs* act as regulators in crucial cellular events like cell proliferation, cell differentiation, and cell death during normal development. Genomic studies of *C. elegans* and *Drosophila* suggested that *miRNAs* have a crucial role in regulating cancer-relevant cellular phenotypes (Hwang and Mendell, 2006). *miRNAs* influence apoptosis either as an anti-apoptotic molecule (targeting pro-apoptotic mRNAs) or as tumor-suppressors (by targeting anti-apoptotic mRNAs) (Lima *et al.*, 2011). Likewise, some *miRNAs* function as oncogenes, while others behave as tumor suppressor genes in a cell-typed manner (Mohamed, 2017). *miRNAs* with pro-proliferative and anti-apoptotic activity likely promote oncogenesis and thus may be overexpressed in cancer cells.

Abnormal *miRNA* expressions were observed to be very common in malignancies. In the study on Chronic Lymphocytic Leukemia (B-CLL), Cimmino *et al.* (2005) had found that the most common chromosomal abnormality in this disorder was the deletion of chromosome 13q14. They also found that the tumor suppressor activity was imparted by *miRNAs* (specifically *miR-15a* & *miR-16-1*) as they were localized in the chromosomal deleted regions in B-CLL patients. They concluded that, in the human genome, *miRNAs* were mostly located at sites that are frequently amplified, deleted, or rearranged in cancer. Dysregulation of *miRNA* expression was observed in other cancer types as well, such as Burkitt's lymphoma, colorectal cancer, lung cancer, breast cancer, and glioblastoma (Hwang and Mendell, 2006).

miRNAs are targets of the *MYC* gene; it has been established that *MYC* regulates the expression of many *miRNAs* leading to their repression. Concurrently, *MYC* is also regulated by *miRNAs*, and the combined effect of sustained *MYC* expression and repressed *miRNAs* regulates oncogenesis by *MYC* (Chang *et al.*, 2008). For instance, *MYC* up-regulates expression of a set of *miRNAs* (*miR-17-92* clusters) (O'Donnell *et al.*, 2005; Mohamed, 2017); from this cluster, *miR-19* was identified as the key oncogenic factor (Wang *et al.*, 2014), which is commonly amplified in several human cancers such as carcinomas, medulloblastomas, neuroblastomas, B-cell lymphoma, and several subtypes of aggressive lymphomas, thus suggesting that *miR-17-92* might contribute to the oncogenic properties of *MYC*.

miRNAs also display their oncogenic effect by inhibiting apoptosis, which is attained by down-regulation of *TP53*, *E2F1* (*E2 Factor transcriptional factor 1*), and *PTEN* (*Phosphate and Tensin homolog*), leading to the activation of *AKT* (protein kinase B) pathway and eventual inhibition of apoptosis (Lima *et al.*, 2011). The onset of cancer in *eu-MYC* B-cell lymphoma can be dramatically accelerated by the insertion of a truncated *miR-17* cluster (*eu-MYC* transgenic mice develop B-cell lymphoma late in life). It is also found that *miRNAs* expressing lymphomas show a high mitotic index with lesser apoptosis (Wang *et al.*, 2014). A decreased level of apoptosis was observed in the *eu-MYC miRNA*-expressing tumors. *E2F1* is a pro-apoptotic factor, which is

transcriptionally activated by *c-MYC* (Fernandez *et al.*, 2003); at the same time, its translational efficiency is compromised by *miR-17*. This simultaneous effect of *c-MYC* and *miR-17* modulates the activity of E2F1 to disapprove apoptosis and favors proliferation (Coller *et al.*, 2007). *miR-21* is another member of the family displaying anti-apoptotic activity; overexpression of this *miRNA* was observed in glioblastoma tumor cell-lines (Chan *et al.*, 2005) and breast cancer tissue (Kumar *et al.*, 2013).

Engulfment gene mutations

The term engulfment is used to describe the removal of apoptotic cells (Hsu and Wu, 2010). An instant removal of apoptotic cells is very crucial to ensure that cells induced to undergo apoptosis must die rather than recovering themselves after the initial stages of apoptosis. Engulfment of an apoptotic cell is different from phagocytosis as it does not evoke inflammatory response because cells being removed carry self-antigens; therefore, apoptosis is commonly termed as “immunologically silent” cell death.

Apoptotic clearance is a complex process accomplished in multiple steps with the help of abundant surface receptors and signaling molecules. The “find-me” signals sent by the apoptotic cells are sensed by the engulfing cells which recognize the “eat-me” signals on the apoptotic cell surface; this leads to intracellular signaling, causing engulfing cup formation around the apoptotic cell which is then ingested before its processing (Ravichandran and Lorenz, 2007; Elliott and Ravichandran, 2010). Administration of antitumor chemotherapeutics mostly induces apoptosis of tumor cells, efficient engulfment of apoptotic cells, and the characteristic release of anti-inflammatory mediators such as TGF β (the antitumor immune response is suppressed by the encounter of TGF β with eat-me signals) (Fond and Ravichandran, 2016).

Mutations in engulfment genes enhance the frequency of cell survival. It appears feasible that apoptotic cell clearance could have a profound impact on carcinogenesis, but there are very few genetic studies to prove the specific role of engulfment signaling pathways in tumorigenesis. Although engulfment of apoptotic cells has been extensively studied, much less is known about how mutations in engulfment genes provide a proliferative advantage to dying apoptotic cells and how do they assist cancer progression. With the recent discoveries that apoptotic cells release many “find-me” factors that attract phagocytes towards apoptotic cells, new insights have been gained on the connection of cell clearance with tumorigenesis (Elliott and Ravichandran, 2010).

Studies on Burkitt lymphoma determined how macrophages sense apoptotic tumor cells and eventually engulf them, and how this mechanism impacts tumor progression (Truman *et al.*, 2017; Ogden *et al.*, 2019). By studying fractalkine receptor-deficient mice, Truman *et al.* (2017) found that fractalkine is a potent macrophage attractant as they observed overexpression of fractalkine in neoplastic B cells. However, Ogden *et al.* (2019) found that macrophages produce IL-10 that seems to suppress tumor immunity, and simultaneously release B-cell survival factor that appears to promote tumor growth. Few studies reported that chronic inflammation is a key factor in tumorigenesis,

but the removal of apoptotic cells does not elicit an immune response; hence, its role in cancer progression remains mysterious (Condeelis and Pollard, 2006).

Post translational modification

Cancer cells also employ (or distort the regular) post-translational modifications of apoptotic regulatory proteins to evade apoptosis. Either functions or stability (or both) of anti-apoptotic and/ or pro-apoptotic proteins are altered by post-translational modification processes such as phosphorylation and ubiquitination (Dai and Gu, 2010; Vucic *et al.*, 2011; Kim *et al.*, 2012). Kinases and acetyltransferases promote the post-translational modification of apoptotic modulators (Declercq *et al.*, 2009).

Post-translational modification ubiquitination/ubiquitylation modifies protein by adding ubiquitin; it can be mono- or poly-ubiquitination (depending on the number of ubiquitin molecules linked) (Vucic *et al.*, 2011). Ubiquitination plays important role in cellular processes like cell cycle regulation, and is a step-wise sequential action of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3); each catalyzing transfer of covalent bond with ubiquitin, from one enzyme to other until it reaches the target protein. Improper ubiquitination affects protein degradation, sequentially causing protein accumulation, deregulated apoptosis, and cancer (Vucic *et al.*, 2011).

Following apoptotic stimuli, the MCL-1 (anti-apoptotic) level drops, which accelerates MOMP. So far, studies have found five E3 ligases that ubiquitinate MCL-1 for degradation. BAX is an important pro-apoptotic BCL-2 family member, which has a direct impact on MOMP. In response to apoptotic stimuli, BAX moves towards the mitochondrial outer membrane to initiate MOMP. The recently identified p53RFP ligase (one of the E3 ligases) was found to ubiquitinate BAX on the mitochondrial outer membrane, leading to its degradation (Benard *et al.*, 2010). Degradation of BAX is linked with high tumor grades in prostate cancer (Agrawal *et al.*, 2008).

Phosphorylation is the most prominent post-translational modification, which can promote protein folding to improve stability as well as serve regulatory functions. Phosphorylation is found to be most frequently damaged in cancer. By phosphorylation of anti-apoptotic and/or pro-apoptotic proteins, cancer cells modify their functions and prevent apoptosis; or stabilize anti-apoptotic proteins and/or destabilize pro-apoptotic proteins to inhibit apoptosis. Phosphorylation can also influence ubiquitination by enabling a docking site for ubiquitin-E3-ligase (Kaleigh and Kurokawa, 2013).

In cancer cells, apoptosis is also down-regulated by inhibiting activation of APAF-1 by phosphorylation; for example, in prostate cancer, RSK (Ribosomal S6 Kinase) is overexpressed and it phosphorylates APAF-1, resulting in inhibition of its activation (Kim *et al.*, 2012; Clark *et al.*, 2005). Phosphorylation can alter the function of apoptotic regulators; if phosphorylation is carried out by a pro-apoptotic kinase JNK (c-Jun N-terminal Kinase), pro-apoptotic proteins are activated and anti-apoptotic proteins are inhibited; conversely, if phosphorylation is done by a

pro-survival kinase AKT or ERK, pro-apoptotic proteins are inhibited and anti-apoptotic proteins are activated. Phosphorylation also plays a key role in the stability of MCL-1 protein. Phosphorylation of MCL-1 by JNK occurs at the T163 site, followed by other sites S155/ S159, eventually leading to proteasomal degradation of MCL-1. On the contrary, phosphorylation by AKT or ERK (Extracellular signal Regulated Kinase) occurs only at the T163 site imparting substantial stabilization to MCL-1. As the AKT/ERK level is high in cancer cells, they phosphorylate MCL-1 at only T163 (leading to MCL-1 stabilization), whereas in normal cells, the AKT level is lowered and JNK is activated, which phosphorylates MCL-1 at S155/S159/T163 and causes degradation of MCL-1 (Lei and Davis, 2003; Maurer *et al.*, 2006; Kopper and Peták, 2008; Nifoussi *et al.*, 2012). Similarly, phosphorylation of BIM by JNK promotes the pro-apoptotic activity of BIM, while ERK phosphorylation of BIM prevents the interaction between BIM and BAX, thereby inhibiting apoptosis (Kaleigh and Kurokawa, 2013).

The serine-threonine kinase AKT is also found to phosphorylate pro-apoptotic protein, thereby inhibiting apoptosis and exerting survival advantage to the cell (Datta *et al.*, 1997). The BH-3 only protein PMAIP1 (Phorbol-12-myristate-13-Acetate-Induced Protein 1 - also known as Noxa), is a pro-apoptotic protein that interacts with MCL-1 and supports its degradation. In cancer cells where higher glucose levels are observed, CDK5 (cyclin-dependent kinase) is activated, which phosphorylates Noxa at Ser13, thereby stabilizing Noxa and suppressing its pro-apoptotic activity (Alves *et al.*, 2006). Phosphorylation of BAD inhibits its pro-apoptotic function. When BAD is phosphorylated at Ser112, Ser136, and Ser155 sites, it provides a docking site for adaptor protein 14-3-3. The binding of adaptor protein at this site prevents the interactivity of BAD and BCL-XL, which results in a lack of MOMP, leading to apoptosis inhibition (Zha *et al.*, 1996). Even caspases involved in apoptosis can be suppressed by their direct phosphorylation (Seifert and Clarke, 2009), or substrates of these caspases are phosphorylated, which protects these substrates from proteolytic cleavage by caspases, e.g., phosphorylation of BID by CK1/CK2 (Casein Kinases) protects BID from proteolytic degradation by caspase-8 (Kurokawa *et al.*, 2008).

Metabolic alterations

Some tumor cells use altered metabolism compared to normal cells, as they need to fulfill their nutrient requirement (to build new cells) from nutrient-deprived surroundings. However, recent studies suggested that altered metabolism is one of the ways cancer cells avoid apoptosis, as metabolic alterations modulate apoptotic machinery to protect the cell from death (Heiden *et al.*, 2009; Matsuura *et al.*, 2016; Pavlova and Thompson, 2016).

Tumor cells adapt their metabolism to their microenvironment and take up more biomolecules (such as glucose, lipids, nucleotides, amino acids) to meet the requirements of rapidly dividing cells. Thus, glucose uptake is intensified in cancer cells, and unlike normal cells (which depend on oxidative phosphorylation for energy generation), cancer cells ferment the glucose to lactate by

aerobic glycolysis, even in presence of an ample amount of oxygen. This is called as “Warburg effect” (Heiden *et al.*, 2009), and also enhanced anaerobic glycolysis is observed in cancer cells (Matsuura *et al.*, 2016). Cellular metabolites such as glucose, lipids, amino acids, and nucleic acids; are found to regulate functions of pro-apoptotic and anti-apoptotic proteins (Kaleigh and Kurokawa, 2013). Among the metabolic pathways, glucose and lipid metabolism are specifically altered in cancer cells, which can impact the apoptotic pathways (Matsuura *et al.*, 2016).

Glucose taken up by cells is converted to Glucose-6-phosphate with the help of hexokinases, which further undergoes either glycolysis or pentose phosphate pathway (PPP). If it undergoes glycolysis, energy is generated (32 ATP molecules) at the end via Kreb’s cycle and oxidative phosphorylation, whereas PPP generates fatty acids, ribose-5-phosphate (precursor for nucleotide), and NADPH (Nicotinamide Adenine Dinucleotide Phosphate). This is why PPP is considered to be essential for biomass production; as cancer cells (being highly proliferative cells) require much biomass, PPP is enhanced in cancer cells to meet their requirement. Solid tumor development increases the cell-density, which creates hypoxic conditions (low oxygen) due to which oxidative phosphorylation is suppressed and PPP is enhanced. In hypoxic conditions, gene expression is regulated by HIF-1 (Hypoxia-Inducible Factor-1), which is overexpressed in several types of cancers (Semenza, 2002). HIF-1 up-regulates its target genes, and many of these gene products encourage glycolysis. Studies showed the correlation between HIF-1 activity and tumor formation (Unruh *et al.*, 2003; Fulda and Debatin, 2007). Besides, HIF-1 suppresses pro-apoptotic protein BID (Seenath *et al.*, 2008).

AKT activated in cancer cells increases glycolysis and lactate production (Elstrom *et al.*, 2004). AKT promotes the union of outer mitochondrial membrane and hexokinase in presence of glucose, which inhibits the release of cytochrome-c (Majewski *et al.*, 2004). Moreover, AKT down-regulates MCL-1 degradation (discussed under phosphorylation section). In presence of glucose AKT suppresses the function of p53 (Yang *et al.*, 2006), which indicates that AKT conceals PUMA induction in presence of glucose. Pro-apoptotic protein BAD is also a substrate for AKT (Datta *et al.*, 1997).

p53 also plays role in cellular metabolism (Bensaad *et al.*, 2006); the p53 protein represses the transcription of the *GLUT1* (Dang, 1999), and loss of p53 activity leads to the Warburg effect (Levine and Puzio-Ku, 2010). *TIGAR* (*TP53*-Induced Glycolysis & Apoptosis Regulator) is a p53 target gene, which regulates glucose metabolism; it lowers the intracellular concentrations of fructose 2,6 bisphosphate and decreases glycolysis by switching to the PPP (Bensaad *et al.*, 2006). *TIGAR* also has functional similarities to the bisphosphate domain of PFK-2/FBPase-2 (Phosphofructo Kinase-2/ Fructose Biphosphatase-2) in regulating glycolysis, and it protects the cell from ROS (Reactive Oxygen Species)-associated apoptosis (Levine and Puzio-Ku, 2010; Dai and Gu, 2010). As p53 is genetically mutated or functionally inactivated in the majority of cancers, these cells are expected to become highly glycolytic following loss

of normal p53 activity (Muller and Vousden, 2013). Therefore, p53-mediated glucose metabolism is likely dysregulated in most cancers.

Glucose metabolism regulates Noxa phosphorylation (Lowman et al., 2010). In cancer cells, higher glucose levels aid in the phosphorylation of Noxa, leading to suppression of its pro-apoptotic activity (discussed in phosphorylation). NADPH produced in PPP contributes to metabolic inhibition of apoptosis by controlling the redox state of cytochrome-c, as reduced cytochrome cannot form apoptosome. However, oxidized cytochrome-c (oxidized by ROS) forms apoptosome. This shows that glucose metabolism negatively regulates apoptosis by producing NADPH (Chandra et al., 2006). Cancer cells alter metabolism to make maximum use of available elements to synthesize macromolecules needed in large amounts in rapidly proliferating cells; for example, catabolism and disposal of nitrogen and carbon are altered in cancer cells (Keshet et al., 2018). As in the urea cycle, enzymes ASL (Argininosuccinate Lyase) and ASS1 (Argininosuccinate Synthase-1) are altered, which leads to the accumulation of ornithine that is used to produce polyamines. Polyamines are nitrogenous compounds that can inhibit apoptosis and assist tumor progression. Even arginine acts as a precursor for polyamines in the tumor microenvironment (Pavlova and Thompson, 2016).

Ca⁺⁺ also plays role in apoptosis and cancer progression. When there is a high concentration of Ca⁺⁺ in ER, Ca⁺⁺ are released from ER (Endoplasmic Reticulum) due to apoptotic stimuli; consequently, the level of Ca⁺⁺ in mitochondria increases, which increases the permeability of the mitochondrial membrane and facilitates the flow of pro-apoptotic molecules into the cytoplasm (Chami et al., 2004; Jeong and Seol, 2008). Recent studies suggest that the connecting points between ER and mitochondria are the target sites for oncogenes and tumor suppressor genes. This Ca⁺⁺ flow between ER and mitochondria determines the metabolism of cancer cells (Pedriali et al., 2017).

Mitochondrial metabolic alterations have been observed in cancer cells; alteration of the physiological mitochondrial metabolism might act as an oncogenic trigger (details under "Mitochondrial alterations").

Mitochondrial alterations

Recent studies have found that in most of the cancer cells, mitochondria drive the malignant phenotype (Riganti and Donadelli, 2019). As mentioned earlier, mitochondria play an important role in apoptosis.

In metabolic aspects of apoptosis, mitochondria provide energy for the apoptosis. As cancer cells are dividing rapidly, they need energy, and mitochondria are energy suppliers of the cell, hence in cancer cells, mitochondrial functions are accelerated. For example, mitochondria-related metabolic pathways are exploited to produce ATP and building blocks, mitochondrial ROS functions as signaling molecules for anti-apoptotic pathways. Alterations of specific mitochondrial enzymes exert oncogenic properties e.g. SDH (succinate dehydrogenase) loses its function in cancer cells because of inactivating mutations due to which its substrate succinate (oncometabolite) gets accumulated that promotes

cell proliferation (Dalla Pozza et al., 2019). Hence loss in function of SDH aids cell proliferation, whereas overexpression of SDH suppresses cell proliferation and promotes apoptosis (Dong et al., 2008). Similarly, FH (fumarate hydratase) function is lost in cancer cells leading to the concentration of its substrate fumarate. How FH reduction leads to cancer is still elusive (Riganti and Donadelli, 2019).

Mitochondria have an essential role in mediating intrinsic apoptosis induced by diverse stimuli. In cancer cells, mitochondrial apoptosis is inhibited either by obstructing MOMP (by affecting BCL-2 family proteins and/or by loss of p53 function) or by inactivating caspases (by suppressing Apoptosome with the help of inhibitory phosphorylation of APAF-1, or by cytochrome c ubiquitination) (Lopez and Tait, 2015). As mitochondria play a substantial role in apoptosis, defects of *mtDNA* might have the capacity to alter cellular response towards apoptotic stimuli, which might provide a survival advantage to the cell. Studies have observed *mtDNA* mutations in several cancers, such as breast cancer, ovarian cancer, colorectal cancer, gastric cancer, hepatic cancer, esophageal cancer, pancreatic cancer, prostate cancer, thyroid cancer, renal cell carcinoma, hematologic malignancies, brain tumor, and other solid tumors (Carew and Huang, 2002). Also, the nuclei-encoded long non-coding RNA MEG3 (Maternally Expressed 3) was found in mitochondria enhancing apoptosis by reducing the expression of Bcl-2 and promoting the release of Cyt-c to the cytoplasm (Wang et al., 2015). *ncRNAs* (Non-coding RNAs) like *ASncmtRNA-1* and *ASncmtRNA-2* were found inducing apoptosis in several human and mouse tumor cell lines and involved in aging and replicative senescence in normal human cells (Landerer et al., 2011).

According to Chinnery and Turnbull (2000), different levels of heteroplasmy in *mtDNA* (within a cell, mitochondria with wild-*mtDNA* and mitochondria with mutant *mtDNA* co-exist) might have a significant importance in the expression of *mtDNA* genes and nuclear genes, including genes involved directly and indirectly in apoptosis and tumorigenesis. According to the studies on several diseases from a mito-epigenetic point of view, abnormal *mtDNA* methylation is associated with pathological conditions, and minute alterations in these *ncRNAs* might affect their apoptosis-inducing ability. It has been established that both intrinsic and extrinsic pathways require mitochondria in certain cell types, where death receptor proteins interact with mitochondria (Khosravi-Far, 2004; Zeng et al., 2012).

Escaping immune system scrutiny

Interactions between the immune system and malignant cells play an important role in tumorigenesis. Many mechanisms of tumor resistance to immune rejection have been studied, but very few of them involve immune escape by using alteration in apoptosis. As already mentioned, tumor cells somehow alter the expression of molecules involved in apoptosis signaling, which can result in resistance of tumor cells to immune-rejection (e.g., mutations in surface receptors like DR5 or FAS); paradoxically, molecules with altered expression

might also serve as antigens of tumor cells (e.g., overexpressed non-mutated p53 in tumor cells may act as tumor antigen for T-cell) (Lee Peter *et al.*, 1999).

Another strategy used by tumor cells to evade anti-tumor immunological response is by inducing apoptosis of host lymphocytes, termed as “tumor counterattack” (Igney and Krammer, 2002; Horton and Gajewski, 2018); for instance, in colon cancer, soluble mediator sFASl (released by membrane-bound FASl on colon cancer cell) induces apoptosis of host lymphocytes and allows tumor progression (Song *et al.*, 2001). However, the induction of apoptosis by sFASl is not clearly understood and hence needs further elucidation.

Macrophages constitute the immune system, which forage for apoptotic bodies. They can induce apoptosis (Diez-Roux and Lang, 1997) and in most cases, macrophage cytotoxicity results in the death of target cells through apoptosis (Aliprantis *et al.*, 1996). In an attempt to treat treatment-resistant tumor cells, Whitworth *et al.* (1990) suggested that systematic activation of tumoricidal properties of the host’s macrophages could help eliminate such resistant tumor cells. A study on laboratory animals clearly indicated that inhibition/elimination of macrophages disrupts apoptosis (Diez-Roux and Lang, 1997). Thus, it demonstrates that macrophages play important role in completing the apoptosis process, and inhibition or elimination of macrophages may hinder apoptosis.

Epigenetics

Epigenetic modifications are DNA modifications that do not change the DNA sequence but affect gene activity. Epigenetics is greatly involved in gene dysregulation in cancer cells. Even mutation-free cells can be induced with pro-cancer characteristics, due to epigenetic changes (Sarkar *et al.*, 2013). Progressive epigenetic alterations in advancing tumors result in an aberrant apoptotic pathway, thereby promoting tumor development (Pal *et al.*, 2010). Epigenetics affects the pro- and anti-apoptotic protein expressions and provides apoptosis resistance to cancer cells (Hervouet *et al.*, 2013). Recent progress in cancer epigenetics found modifications such as DNA methylation and hydroxymethylation, histone acetylation and methylation, nucleosome positioning, and changes in small *ncRNAs* and *miRNAs* (Sarkar *et al.*, 2013; Qu *et al.*, 2013). The most widely focused epigenetic changes in cancer are DNA methylation. Evidence suggests that the downregulation of apoptotic genes due to DNA methylation (e.g., aberrant DNA methylation patterns, hypermethylation, and hypomethylation) is one of the reasons for cancer cells to evade apoptosis (Hervouet *et al.*, 2013; Gopisetty *et al.*, 2006).

Methylation

DNA methylation confers stable gene silencing thus plays important role in gene expression regulation and chromatin architecture (Qu *et al.*, 2013). Methylation plays a crucial role in tumorigenesis. The DNA methylation results from a transfer of a CH₃ (methyl group) from S-adenosyl methionine to the fifth carbon of cytosine in CpG (Cytosine triphosphate deoxynucleotide-phosphodiester bond-Guanine triphosphate deoxynucleotide) motifs. It occurs in about

50% CpG in the genome; the CpG-rich areas, referred to as CpG islands (CGI), are more frequently methylated than isolated CpG (Hervouet *et al.*, 2013). DNA methylation between cell division is maintained by DNMT1 (DNA-methyltransferases 1) that is found to be highly expressed in cancer cells (Robertson, 2000). As reported, DNA methylation occurs either after replication on neosynthesized strands of DNA by DNMT1, or *de novo* on unmethylated DNA strands by DNMT3a and DNMT3b (Chen *et al.*, 2003; Bird, 2002). Several apoptosis-linked genes are silenced by methylation, such as *APAF1*, *p14*, *BCL2*, *EDNRB* (*Endothelin Receptor B*), in bladder cancer *DAPK* (*Death Associated Protein Kinase*), *PUMA* (in lymphoma), *FADD*, *TNFRSF25* (*TNF Receptor Superfamily member 25*), *TNFRSF21* (*TNF Receptor Superfamily member 21*), *TRAILR3* (*TNF Receptor Superfamily member 10c*), *LITAF* (*Lipopolysaccharide Induced TNF Factor*), *BAX*, *CASP8*, *CASP3*, *CASP9*, *DR4* (*Death Receptors 4*), *DR5*; cell adherence *CDH1* (*Cadherin-1*), *CDH3* (*Cadherin-3*); tumor suppressor *p53*, *RASSF1A* (*Ras Associated Domain Family-1 isoform A*), *BLU*; cell cycle regulator *CHFR* (*Checkpoint protein with Fork-head and Ring-finger domain*), *p16*, *p21*, *p27*; DNA repair *MGMT* (*Methyl Guanine Methyl Transferase*), *ARH1* (*ADP-Ribosylarginine Hydrolase-1*) (breast and ovarian cancer); cellular growth suppressor *RIL* (*Reversion-Induced LIM domain*); and *TMS1* (*Target of Methylation-induced Silencing*)/*ASC* (*Apoptosis associated Speck-like protein containing a CARD*); each gene harbors a CpG island in its 5’ region (Hervouet *et al.*, 2013; Friedrich *et al.*, 2004; Martinez *et al.*, 2007; Sarkar *et al.*, 2013; Das *et al.*, 2006; Boumber *et al.*, 2007). The Knudson’s “two-hit hypothesis” proposes promoter methylation as a pathway of carcinogenesis (Jones and Laird, 1999). The promoters of TSG (tumor suppressor gene) in cancer cells are silenced epigenetically, which leads to tumor progression through apoptosis inhibition (Sarkar *et al.*, 2013). It is important to note that methylation of CpG sites after transcription of initiation site does not block expression; thus, it may not lead to gene silencing, but it can be used as a tumor marker.

Aberrant methylation: Transcriptional silencing of TSG promoters by aberrant methylation is another way of cancer development (Das *et al.*, 2006), and this is found to be a hallmark of gastric cancer (Qu *et al.*, 2013). Expression of silenced TSGs can be regained by demethylation, which results in cell cycle inhibition and apoptosis (Mataga *et al.*, 2012). Usually, aberrant methylation leads to loss of gene function, thus providing survival advantage to neoplastic cells (Jones and Baylin, 2002). DNA coding for *miRNAs* also undergoes aberrant methylation, causing abnormal levels of *miRNAs* in various cancers from solid tumors to blood cancers (Lujambio *et al.*, 2007).

Hyper-methylation: DNA hypermethylation carries out pro-apoptotic gene silencing (Hatziapostolou and Iliopoulos, 2011). Many apoptotic genes were found to be hypermethylated in cancer cells; few are more specific to tumor type, while others are found to be commonly hypermethylated in cancer (Garrison *et al.*, 2008). Hyper-methylation of the TSG promoter is the prominent epigenetic change in human neoplasia (Friedrich *et al.*, 2004). In ovarian cancer, decreased expression of *TRAIL*

due to hypermethylation was observed (Horak *et al.*, 2005). Hypermethylation of apoptosis-related genes covers large promoter sequences, and it is found to be irregular (Mohammad *et al.*, 2015). Further study is needed to design demethylations therapies to treat hypermethylation-dependent silencing of TSGs.

Hypomethylation: Hypomethylation abnormally activates oncogenes. DNA hypomethylation plays important role in tumor formation and progression (Ehrlich, 2009). However, less is known about apoptotic genes hypomethylation in cancer.

Cancer cell dormancy

Tumor dormancy came into light when disease relapse was observed in several cancer patients, years after their surgical treatment. Tumor dormancy is crucial in understanding the metastatic disease. It is contrived that few cancer cells survive even after the supposedly successful treatment. These residual cancer cells may dwell in distant organs and contribute to disease recurrence; these are termed as disseminated tumor cells (DTCs), and a subgroup of DTCs circulating in the blood is termed as circulating tumor cells (CTCs) (Mohme *et al.*, 2017; Gao *et al.*, 2017). DTCs and CTCs get exposed to diverse microenvironments during dissemination and circulation, which constrains tumor growth; moreover, when they arrive on a secondary site, they must undergo apoptosis, but they maintain long-term survival. The key mechanism behind this resistance is found to be tumor dormancy (Townson and Chambers, 2006).

Dormancy can be of two types, dormant solitary cells and dormant micrometastases (Naumov *et al.*, 2001; Naumov *et al.*, 2002). In dormant micrometastases, there is a balance between proliferation and apoptosis, resulting in no net increase in tumor size; this may be due to angiogenic/immunologic dormancy (Kareva, 2016). On the other hand, dormant solitary cells are quiescent cells with no proliferation and apoptosis; this might be due to G0-G1 arrest, or they might release certain factors and modulate growth-related signaling pathways to accommodate themselves in the new microenvironment (Gao *et al.*, 2017; Aguirre-Ghiso, 2007). However, they can exit dormancy and proliferate again, yet the clear mechanism as to how is it decided is unknown, which cell would continue to be dormant, which would undergo apoptosis, and which would start proliferating (Kareva, 2016). Thus, these dormant cells are potentially resistant to therapies that rely on cell cycle progression and apoptosis induction (Naumov *et al.*, 2003). Studies were reported on detecting DTCs (in the bone marrow and lymph node) and CTCs (in the blood) in asymptomatic patients at the early stage so that it could be used as selection markers and monitoring tools (Mohme *et al.*, 2017; Lianidou *et al.*, 2015).

It is highly conceived that solitary dormant cells are responsible for tumor recurrence, but the clear mechanism is yet to be elucidated, regarding how this dormant cell localizes in the tissue for a long time, how it remains unnoticed by the immune system, and how does it give rise to large metastasis.

Cancer clonal theory and stem cells

Regarding the origin of cancer, the two basic theories being considered are cancer clonal theory and cancer stem cells

(CSCs) hypothesis. According to the cancer clonal theory, random mutations lead to malignant transformation and subsequent clonal selection of cancer cells with the potential to regenerate tumor growth (Tysnes, 2010). The clonal theory is being widely accepted and is gaining attention for the development of therapies that attack individual clones with genetic survival traits. One of the areas of these beneficial mutations was found to be in apoptotic genes (Messerschmidt *et al.*, 2017).

The CSCs hypothesis suggests that tumor arises from a small population of self-renewing stem-like cells with unlimited proliferation potential, which are dissimilar to differentiated progeny (Reya *et al.*, 2001; Garcia *et al.*, 2012). Both theories are acceptable, and it is worth noting that these two paradigms are not disparate, as CSCs go through clonal evolution (Barabé *et al.*, 2007). The CSC model is currently well-established in the context of developing therapies. Studies suggest that CSCs are disseminated tumor cells (DTCs) holding stem cell-like phenotype, and they are dormant cells causing metastasis and relapse (Chaffer and Weinberg, 2011; Mascré *et al.*, 2012; Naik *et al.*, 2016; Kleffel and Schatton, 2013). The cancer stem cells' apoptosis resistance may involve innate cellular mechanisms and extrinsic factors such as self-renewal, differentiation pathways (e.g., Wnt, Notch, and Hedgehog), tumor suppressor genes (*PTEN*, and *TP53*), asymmetric cell division, indefinite self-replication, genetic instability, epigenetics, chromatin changes, mobilization, and modified microenvironment (e.g., secreted survival factors, adhesion-mediated apoptosis resistance, and hypoxic conditions) (Barabé *et al.*, 2007; Medina *et al.*, 2009; Krut and Schuringa, 2010; Garcia *et al.*, 2012). Overexpression of anti-apoptotic BCL-2 molecules is associated with CSCs (Garcia *et al.*, 2012). Upregulation of ABC (ATP-Binding Cassette) transporters confers resistance to toxic agents and multi-drugs (Tysnes, 2010; Fulda and Pervaiz, 2010). In certain types of cancers, CSCs showed elevated expression of death receptors such as DR4 (in colon cancer) and DR5 (in bladder cancer) (Signore *et al.*, 2013; Sussman *et al.*, 2007; Szliszka *et al.*, 2009). CSCs are found to be TRAIL-resistant due to overexpressed c-FLIP (cellular FLICE Inhibitory Protein) (Piggott *et al.*, 2013). Certain miRNAs are found to have an impact on CSCs fate; for example, *miR-34* suppresses CSCs tumor formation by apoptosis regulation (Ji *et al.*, 2009).

These studies are yet under investigation and need rigorous research, as the possibility of dedifferentiation of differentiated cells into CSCs cannot be denied. Evidence is needed to prove tumor formation from early progenitors (normal stem cells) (Krut and Schuringa, 2010).

Reversibility of apoptosis

It has already been mentioned that MOMP might be a point of no return, but cells could survive MOMP in few situations (Lopez and Tait, 2015). The early stages of apoptosis are reversible upon removal of the apoptotic stimulus. Many studies found that when apoptosis is induced in cancer cells via chemotherapy, apoptosis is reversed after initial response and the cancer cells are returned back; this is due to inefficient apoptosis (Geske *et al.*, 2001; Kim and Tannock, 2005;

Letai, 2008; Tang *et al.*, 2009). Tang *et al.* (2009) showed that when various cancer cell lines (cervical carcinoma, skin cancer, liver cancer, breast cancer, prostate cancer) were exposed to apoptotic inducers (e.g., jasplakinolide, ethanol, staurosporine), they started to undergo apoptosis and displayed apoptotic features (such as mitochondrial dysfunction and fragmentation, nuclear condensation, cell shrinkage, and caspase activation); however, upon removal of these inducers, they reverted back apoptosis and survived. This study also found that these features of apoptosis were not point-of-no-return, as apoptosis could reverse even after these stages; however, it could not be reversed after nuclear fragmentation. According to Geske *et al.* (2001), DNA repair is activated early in the p53-induced apoptotic process, which might be involved in reversing the cell death pathway in some circumstances.

Summary and future challenges

It is obvious from the present review that cancer cells apply multiple mechanisms to evade apoptosis, which influence cancer progression. Apoptosis is halted by cancer cells using various mechanisms simultaneously, but we cannot exclusively separate any mechanism. Apoptosis can be disturbed by mutation of the genes coding for proteins involved, metabolic alterations, mitochondrial changes, or axing of immunity. These provide a survival advantage to the cell and help cancer development. Few points deduced from the review of extensive research in this field are summarized below:

1. Collectively, the transcriptional network is found to be dysregulated in cancer cells, remarkably affecting the expression of apoptotic regulators, thus resulting in resistance to drugs and favors tumor development (Fig. 3). For instance:
 - Cancer cells modulate BCL-2 and TP53 genes to escape apoptosis, and targeting these genes would be a valuable treatment strategy for a wide variety of tumors.
 - Mutation in caspase genes is one of the ways to escape apoptosis by cancer cells.
 - Cancer cells affect the DR5 receptor to escape apoptosis, which results in tumor cell survival.
 - miRNAs play essential roles in many basic physiologic processes, including apoptosis; thus, abnormalities in miRNA function influence tumorigenesis.

- The expression of several key engulfment players is upregulated in neoplastic cells, but the importance of this observation is unclear.
 - The cell clearance process can influence antitumor immune responses, and apoptotic cell clearance promotes tumorigenesis, or it suppresses tumorigenesis.
 - APAF-1 is the key regulator molecule of apoptosis, and its suppression can mediate tumorigenesis.
2. Post-translational modifications play a critical role in the regulation of apoptosis by modulating apoptotic regulatory protein stabilities and functions.
 3. Metabolic alterations in cancer cells affect apoptosis.
 4. Mitochondria play important role in almost all aspects of apoptosis, such as gene expression regulation, metabolism, and release of pro-apoptotic proteins.
 5. Macrophage cytotoxicity results in apoptosis in the target cell population and could treat cancers. Cancers can be treated by immune therapies that activate macrophages.
 6. Dormant tumor cells are found to resist apoptosis and cause metastasis. Mechanisms through which each cell achieves dormancy might be different.
 7. Reversibility of apoptosis plays role in cancer progression.

As apoptosis is found to be affected in cancers and playing role in tumorigenesis, targeting apoptosis to treat cancer is a valid strategy. However, there are many challenges that need to be addressed, as summarized below:

- Apoptosis-based therapies: Although many therapies targeting apoptosis are already at the stage of human clinical trials, most of the therapeutic agents remain at the pre-clinical stage, due to the lack of their specificity and resistance developed towards them before the completion of treatment. Thus, future studies should focus on identifying the predominant apoptosis evasion mechanism specific to each type of cancer and designing therapeutic agents that can specifically target that mechanism to treat the particular type of cancer.
- Improving the efficacy of existing drugs: Apart from inducing apoptosis in normal cells, the existing drugs are resisted by cancer cells and have side-effects that limit the required dose for treatment. A further detailed study is needed to improve these drugs. As mutations in apoptotic genes also cause resistance,

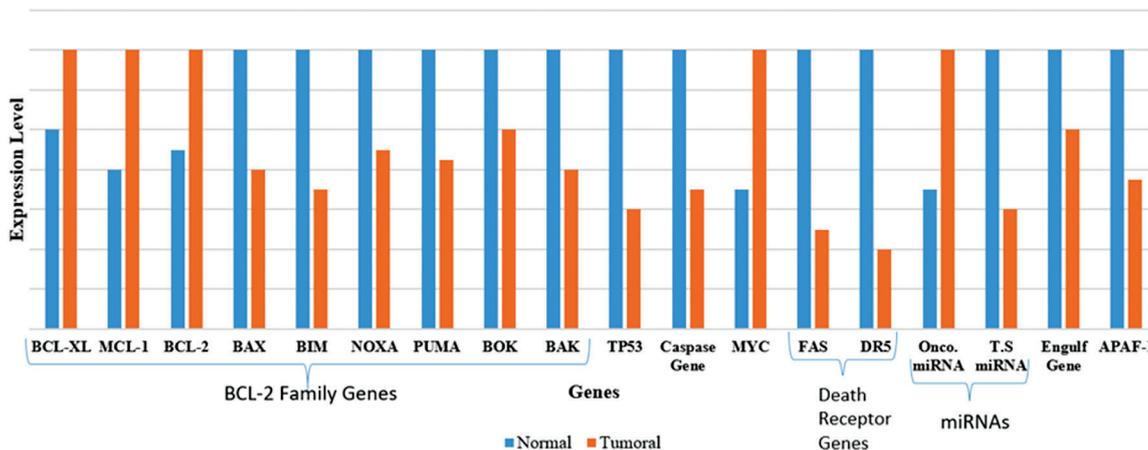


FIGURE 3. Gene expression level in normal and cancer cells (Karbasi *et al.*, 2015).

these mutations must be studied further as they become new therapeutic targets.

- Development of new drugs: Deciphering apoptosis knowledge into clinical practices will be remarkable as it will treat not only cancer but also a broad range of disorders such as Alzheimer's, AIDS, and ischemia-associated injuries. Drugs that modulate either by altering actions of anti-apoptotic and pro-apoptotic proteins or by stumbling RNA transcription (for example by using mimetic of pro-apoptotic transcription factors), holds the potential for use in cancer therapy.
- Apoptotic stimuli in cancer treatment: Stimuli that causes DNA damage in the cell and how the cell responds to the DNA damage play important role in cell death and cell survival. If detailed mechanistic is studied, we can manipulate it to use it in treatments. For example, in p53-induced apoptosis, p53 repairs the DNA damage due to which the cell survives death. What are the factors that decide damage is irreparable and the cell must die? What death pathway to choose; apoptosis or inflammatory response? If how cells take these decisions is known, manipulation of these decisions would be helpful in treatment.
- A detailed study of macrophages: It has been suggested to treat cancer by activating the tumoricidal properties of macrophages. We need to find out a detailed model of macrophage subsets that are actually involved in tumor killing as these subsets possess therapeutic importance.
- Tumor counterattack: Rigorous work is needed to find out the specific and unique antigens displayed by cancer cells so that these antigens can be targeted. Tumor cells display a counterattack which is killing tumor-infiltrating lymphocytes by expressing apoptosis inducing ligand CD95L. Extended research on tumor counterattack is needed to use this phenomenon in treatment.
- Mitochondrial alteration: As mitochondria play important role in apoptosis and aberration in mitochondrial functions facilitates tumorigenesis, many things in mitochondria are yet to be explored; for example, small molecules, used as a sensor in response to different stimuli, must be found out.
- Use of genetic engineering in therapies: As genetic engineering is rapidly advancing, we need to explore the potential means of employing it in cancer treatment, such as introducing anti-oncogene, replacing altered or mutated genes, cutting-off mutated genes, repairing mutation by insertion or deletion of base-pairs.
- Further studies are needed to find out, if genes coding for surface receptors and signaling molecules involved in cell clearance, get mutated because of which cell clearance will fail, then what role this failure will play in tumor progression.
- Metabolic alteration: Further studies are needed to completely elucidate the link between metabolic alteration and apoptosis and its connection to tumorigenesis.

- Immunity escape: Detailed study is required to find how tumor cells alter the immune system to escape apoptosis.
- Cancer dormancy: Rigorous research is needed to explore mechanisms of cancer dormancy and to find specific markers to detect dormant cells, as it has prime clinical importance in metastasis treatment. A clear understanding of chemo-resistant mechanisms by dormant cells might completely change our reflection on tumorigenesis, tumor diagnosis, and their treatment.

Conclusions

A comprehensive review of the various mechanisms used by cancer cells to evade apoptosis is presented. Apoptosis is found to be frequently deregulated in almost all types of cancers studied so far. There has been dramatic progress to explore apoptosis evasion mechanisms by cancer cells, which attracted researchers and health professionals. As the world is facing threats of cancer, there is a growing demand for successful cancer treatment. Killing a cancer cell is the best cancer treatment, for which induction of apoptosis can be a remarkably effective therapy. As most of the compounds that successfully induce apoptosis are usually non-toxic to normal cells, they can be used in cancer therapies. Though many existing cancer therapies treat cancers by inducing apoptosis either by activating apoptotic pathway or by removing apoptosis inhibitors, they are not fully successful with several cancer types and different stages of cancer; moreover, cancer cells gradually develop resistance towards them and block apoptosis induced by these therapeutic agents. The cancer cells' response to these therapies depends on the differences in apoptosis evasion methods and molecular strategies used to escape apoptosis.

Thus, to provide new molecular targets for future therapeutic approaches, a detailed study of apoptosis evasion mechanisms is needed, which involves identifying the escape mechanism predominant in a particular type of cancer and its stage of occurrence. We can develop specifically targeting gene therapies; for example, if it is found that a particular anti-oncogene has been altered to a high extent or lost in a certain type of cancer, introducing oncogene to normal chromosome can be achieved. Moreover, as cancer cells use multiple mechanisms to evade apoptosis, we need agents that can alter these multiple pathways and treat cancer without leaving a single reason for a cancer cell to survive.

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