INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. [1] It is currently the main cause of death in patients with hepatitis C virus (HCV) related cirrhosis, and the issue of HCC in Egypt is extensively increasing. [2] HCC is often diagnosed at advanced stage where effective therapies are lacking, so the surveillance of patients at risk is necessary. [3]

Epidemiology:

HCC has become the third most common malignancy worldwide with very poor prognosis, rendering it the fourth highest cause of cancer-related deaths. [4, 5] The distribution of liver cancer varies by region and more than 80% of cases and deaths occur in developing countries. [6] In Africa, liver cancer has been ranked as the fourth common cancer, and most of liver cancers are HCC. [7] Of all the HCC cases, it is estimated that 66% are attributable to hepatitis B virus (HBV) and 42% are attributable to HCV. [8]

In Egypt, there is a high incidence of HCC as a result of high rate of viral exposures.[9] According to the report of the population-based cancer registry of Gharbiah, the incidence of liver cancer is ranked as the second highest in men and the seventh in women during 2000-2002. [10] Moreover, there has been an alarming increase in incidence of liver cancer in Egypt, which is now three times higher than that in the USA. [11]

Egypt suffers from the world's highest prevalence of HCV infection, where several reports showed seropositivity for HCV ranging from 12.7% in seashore governorate to 36.3% in the Nile delta region of Egypt. [12, 13] Moreover, the intra-familial spread of HCV has been documented as one of the most important routes of transmission, leading to the current estimated burden of infected Egyptians. [14, 15]

Risk factors:

HCV: The HCC epidemic in Egypt is associated with HCV infection; where Egypt has the highest prevalence of HCV in the world. Up to 13.8% of the population is infected and seven millions with chronic HCV related liver diseases was reported. Up to 90% of HCC cases in the Egyptian population were attributed to HCV. Preliminary evidence suggests that genotype 4 HCV infection may place patients at greater risk for HCC than other HCV variants. [16]

The precise mechanism by which HCV infection causes HCC is not known. However, the development of cirrhosis is usually the cause of HCC in HCV infection. [17] It was reported that interferon (IFN) treatment prevents the development of HCC in patients with HCV-related cirrhosis regardless the response status, with stronger preventive effect among sustained responders. [18, 19]

In HCV-infected patients, factors related to host and environment and lifestyle appear more important than viral factors in determining progression to cirrhosis. These factors include: older age overall, older age at HCV acquisition, male sex, heavy alcohol intake, diabetes mellitus, obesity, and co-infection with HIV or HBV. [20]

HBV: 350 million patients all over the world are chronically infected with HBV and this accounts for about ~50% of HCC cases with considerable regional variation. [21] According to Egyptian studies, [13, 14] the prevalence of HBsAg in Egypt is of intermediate endemicity (2–8%). Nearly 2-3 million Egyptians are chronic carriers of HBV. [22] However, the majority (70%-90%) of HBV-related HCC develops on top of cirrhotic livers. [23] Factors which increase risk among HBV carriers include male sex, older age, longer infection duration, family history, aflatoxin exposure, alcohol, tobacco and co-infection with HCV. [24]

Dietary Aflatoxin: Aflatoxins are carcinogenic mycotoxins produced by Aspergillus molds growing on grains, corn, peanuts and fermented soy beans in sub-Saharan Africa and eastern Asia. The active catabolite AFB1 binds to DNA and produces a characteristic mutation in the p53 tumor suppressor gene, which has been detected in 30-60% of HCC tumors in aflatoxin endemic areas. HBV infected individuals exposed to aflatoxin have a higher liver cancer risk, suggesting synergism between both risk factors. Efforts have been done to eliminate aflatoxin exposure of HBV carriers in China and Africa. [25]

Alcohol*:* Heavy alcohol intake (>50-70 g/day for several years) is a well-established HCC risk factor. There is evidence for a synergistic effect of alcohol with HCV or HBV, probably in promoting cirrhosis. [26]

Fatty Liver Disease: Non-alcoholic fatty liver disease (NAFLD), including its more advanced form non-alcoholic steatohepatitis (NASH), has been proposed as the etiological factor for cryptogenic cirrhosis and HCC. However the progression of NAFLD/NASH to cirrhosis and HCC is infrequent. [20] Obesity and diabetes mellitus could be risk factors for HCC development. Up to 90% of all obese individuals body mass index (BMI) >30 kg/m^2 and up to 70% of people with diabetes mellitis have some type of fatty liver. Type 2 diabetes mellitis, has been proposed to be a risk factor for both chronic liver disease and HCC. Several studies found a statistically significant association between HCC and diabetes mellitis. [20]

Diet: The role of diet in the etiology of HCC is largely unknown. However, epidemiological studies previously reported that coffee reduces the risk of developing cirrhosis. Several studies reported a significantly reduced risk of developing HCC with increased coffee consumption. [27]

Clinical presentation:

HCC classically arises and grows in a silent fashion, making its discovery to be prior to the development of later stage disease. The various clinical presentations generally relate to the extent of hepatic reserve at time of diagnosis. Cirrhotic patients tend to have less tolerance for malignant infiltration within the liver and frequently present with signs and symptoms of hepatic decompensation such as jaundice, hepatic encephalopathy, ascites and variceal bleeding. Noncirrhotic patients with HCC typically present in a different manner with Symptoms include malaise, anorexia, wasting, right upper quadrant abdominal pain, and distension. [26] Physical examination may reveal an abdominal mass or hepatomegaly with hard and irregular borders that may demonstrate a vascular bruit. [28] A rare catastrophic complication of HCC is tumor rupture which occurs when a large vascular tumor on the periphery of the liver outgrows its blood supply. These patients

present with sudden severe abdominal pain, peritoneal irritation, and hypotension. Peritoneal lavage or abdominal laparotomy can confirm the diagnosis. [29]

Extrahepatic manifestations of HCC are well described and may be related either to distant metastases or paraneoplastic phenomena. Advanced HCC can metastasize to any organ system via hematogenous or lymphatic routes, and most commonly spreads to bone, lung, and abdominal viscera. [30] Paraneoplastic manifestations may occur in HCC and include hypoglycemia, hypocalcemia, polycythemia, and feminization syndrome. [31] Watery diarrhea has been shown to be significantly more common with HCC on top of cirrhosis than with cirrhosis alone and can be an initial presenting symptom. Increased production of intestinal secretory substances, such as gastrin and vasoactive intestinal peptide (VIP), has been suggested as a possible cause. [26] Moreover, various cutaneous manifestations are well described in HCC including dermatomyositis, pemphigus foliaceus, and pityriasis rotunda, but are not necessarily specific for the disease. [32] Porphyria cutanea tarda (PCT) is frequently associated with chronic HCV and several studies have linked its presence to a higher risk of developing HCC. [33]

Routine surveillance of high-risk patients has made the discovery of asymptomatic HCC more common. These individuals whose tumors are identified prior to the development of hepatic decompensation or other complications described above, are more likely to be better candidates for curative interventions proven to prolong survival. [3]

Pathology and carcinogenesis:

Carcinogenesis of HCC is a multi-factor, multi-step, and complex process. The cellular origin of HCC has long been debated, but whether HCC originates from mature hepatocytes, stem/progenitor cells, or both remain unclear. The fact that many liver tumors arising during cirrhosis when hepatocyte senescence triggers the activation of liver progenitors causes further confusion. [34] In the liver, there may be at least three distinct cell lineages susceptible to neoplastic transformation: hepatocytes, intrahepatic stem cells, and small hepatocytes. [35] Most well-differentiated HCCs in the early stages are detected as small nodules with normal levels of alpha-feto protein (AFP). Subsequently, they increase in size and become moderately or poorly differentiated cancerous tissues producing AFP. [36] It has been suggested that intrahepatic stem cells can give rise to HCC and cholangiocarcinoma (CC). [37] Furthermore, the role of intrahepatic stem cells in carcinogenesis is supported by a histological subtype of liver malignancies that displays features of both HCC and CC or combined (HCC-CC) with the presence of numerous liver progenitor cells. [38]

The development of HCC from premalignant lesions is reported to occur in stages. The regenerative nodules evolve into dysplastic nodules (low and high grade). These may subsequently develop into early HCCs, and if left untreated, become advanced carcinomas.[39]

The gross pathology of HCC is a direct reflection of the imaging findings. HCC may appear as a single mass or as multifocal nodules of variable sizes, and sometimes can be diffusely infiltrative. Macroscopically, small HCCs up to 2 cm in diameter are divided into two types: a distinctly nodular type and an indistinctly nodular type. The classic HCC or the distinctly nodular type is seen as a clear nodule with a fibrous capsule and/or fibrous septa. On the other hand, tumors of the indistinctly nodular type show only a vaguely

nodular appearance with indistinct margins. The tumor is usually paler than normal liver parenchyma. [39]

Microscopically, tumors range from well differentiated to highly anaplastic. The most common histologic pattern is the trabecular pattern, while the scirrhous type is the least common pattern. [39] Histologically; well differentiated HCCs demonstrate distinct trabecular architecture coated by a layer of endothelial cells, which resemble thickened liver cell plates. Poorly differentiated HCCs lose their trabecular architecture and become solid sheets of tumor cells with a haphazard arrangement lacking a distinct endothelial coating. [40]

Staging:

Cancer classification is intended to establish prognosis and enable the selection of the adequate treatment for the best candidates. In addition, it helps researchers to exchange information and design clinical trials with comparable criteria. In patients with HCC, unlike most solid tumors, the coexistence of two life-threatening conditions such as cancer and cirrhosis complicates prognostic assessments. [41, 42] There is no worldwide consensus on the use of any given HCC staging system. However, most major trials of HCC therapy have chosen the Barcelona clinic liver cancer (BCLC) staging system making it the reference staging system. [43]

BCLC staging classification was proposed by Llovet and colleagues in 1999. [44] This classification allows patients to be divided into four different categories based on tumor stage (tumor size, number of nodules, and presence of portal vein thrombosis), liver function (Child-Pugh score), portal hypertension, physical status (performance status), and cancer related symptoms. Furthermore, this staging classification selects the best candidates for the best therapies currently available; as follows: [45, 46]

- Early stage (stage A): includes patients with asymptomatic early tumor suitable for radical therapies as resection, transplantation and percutaneous radiofrequency ablation (RFA) therapy.
- Intermediate stage (stage B): includes patients with asymptomatic multinodular HCC suitable for palliative treatment as transarterial chemoembolization (TACE).
- Advanced stage (stage C): includes patients with symptomatic tumors and/ or invasive tumor pattern in the form of vascular invasion and/ or extra hepatic spread.
- End stage disease (stage D): includes patients with extremely poor prognosis that should receive symptomatic treatment.

Thus, the BCLC has demonstrated superior survival stratification and prognosis prediction over the other classifications. [47] Moreover, BCLC staging is accepted by both the European association for the study of the liver (EASL) and the American association for the study of liver diseases (AASLD), and it is emerging as a standard staging classification in western populations. The most important aspect of this staging classification is that it is linked to an evidence-based treatment algorithm and can easily be used in a clinical setting. [48]

Diagnostic approach to HCC:

Diagnostic Imaging:

Imaging is very important for the diagnosis and staging of HCC. [49] Ultrasound (US) imaging is commonly applied in addition to AFP to help detection of small hepatic tumors <3 cm. Its widespread use as a surveillance tool relates to its noninvasive nature, high availability, and low cost. In combination with AFP the positive predictive value (PPV) can be as high as 94%. However, limitations exist with operator experience and when imaging obese or cirrhotic individuals. [50] The most reliable diagnostic tests are triphasic CT and dynamic MRI, whereas hepatic angiography has fallen out of favor in most practice settings. [49]

Laboratory tests:

Laboratory liver tests are broadly defined as tests useful in the evaluation and treatment of patients with hepatic dysfunction. The liver carries out metabolism of carbohydrate, protein and fats. Some of the enzymes and the end products of the metabolic pathway which are very sensitive for the abnormality may be considered as biochemical marker of liver dysfunction. [51] However, no specific pattern of liver function tests is diagnostic of HCC. [52]

Liver function tests include:

a) Serum Bilirubin: It is the catabolic product of haemoglobin produced within the reticuloendothelial system, released in unconjugated form which enters into the liver and converted to conjugated forms of bilirubin by the enzyme UDP-glucuronyltransferase. [53]

b) Alanine amino transferase (ALT): It is found in small concentrations in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body. ALT is purely cytoplasmic enzyme catalysing the transamination reaction. [53] Despite the association between greatly elevated ALT levels and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage. [51]

c) Aspartate amino transferase (AST): It catalyses transamination reaction. AST exists in two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasmic forms. AST is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney. [53] AST elevations often predominate in patients with cirrhosis and even in liver diseases that typically have an increased ALT. [54]

d) Alkaline phosphatase (ALP): It is present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. It performs lipid transportation in the intestine and calcification in bone. [53] Hepatic and bony metastasis can also cause elevated levels of ALP. Other diseases like infiltrative liver diseases, abscesses, granulomatous liver disease and amyloidosis may cause a rise in ALP. Mildly elevated levels of ALP may be seen in cirrhosis and hepatitis. [51]

e) Gamma Glutamyl Transferase (GGT): It is a microsomal enzyme present in hepatocytes and biliary epithelial cells, renal tubules, pancreas and intestine. Serum GGT activity mainly attributed to hepatobiliary system. [53] Increased level is seen in about 30% of patients with chronic HCV infection. [55] Other conditions like uncomplicated diabetes mellitus, acute pancreatitis, myocardial infarction, anorexia nervosa, hyperthyroidism and obesity may cause elevated levels of GGT. [51]

Tumor markers:

They could be helpful along in patients with HCC; however, there is insufficient data for routine use of most current biomarkers in clinical practice. Therefore, the backbone of early detection, diagnosis and treatment response for HCC remains imaging-based. Alpha fetoprotein is the best studied of all biomarkers and may be of benefit for early detection when used in combination with ultrasound. Several biomarkers of HCC are: [56]

1) Serum alpha fetoprotein (AFP): It is a fetal glycoprotein, synthesized primarily by the embryonic liver, cells of the vitelline sac and the fetal intestinal tract in the first trimester of pregnancy. Thereafter, the serum concentration of AFP declines rapidly after birth and is usually undetectable in adults. [57] The sensitivity and specificity of AFP are not sufficient for early diagnosis, and so additional markers are needed. [58] The development of effective markers for the diagnosis of HCC could have an impact on HCC-related cancer mortality and significant public health implications worldwide. This is an active area of research with several groups reporting new marker candidates within the last few years. [59]

2) Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3): It is produced by malignant liver cells, binds to lectin lens culinaris agglutinin (LCA) with high affinity, and is the major glycoform found in individuals with HCC. [60] AFP-L3 levels ≥10% are associated with up to 7-folds increased risk of developing HCC within the next 21 months and can be elevated 3-21 months before HCC is detected by standard imaging techniques. The diagnostic sensitivity and specificity ranges from 36%-66% and 77%-95% respectively. Because AFP-L3 is produced by malignant hepatocytes, its measurement helps distinguish non-malignant hepatic disease from HCC. Malignant liver cells that produce AFP-L3 have an increased tendency for rapid growth, early invasion, and intra-hepatic metastasis, thus making AFP-L3 an indicator of poor prognosis in affected individuals. [61]

3) Prothrombin induced by vitamin K absence II (PIVKA II), also known as des-gamma carboxyprothrombin (DCP): It is an abnormal prothrombin protein produced as a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in malignant cells. It is also found in patients on warfarin therapy, or with vitamin K deficiency. The defect in carboxylation has been attributed to defective gene expression of gamma-carboxylase in HCC patients. In diagnosis of HCC, serum PIVKA II was found to have a high diagnostic sensitivity and specificity of 48–62% and 81–98%, respectively, in several large case–control studies. [62]

4) Vascular endothelial growth factor (VEGF): It is an endothelial cell mitogen that initiates and promotes neovascularization and endothelial cell proliferation, and it was initially identified as a vascular permeability factor. The expression of VEGF in HCC tissues was correlated with AFP, PIVKA II, tumor size and histological grade of the tumor.

Furthermore, this biomarker was related to invasiveness and metastasis of HCC. The expression of VEGF in HCC patients with microscopic venous invasion was significantly higher than that in HCC patients without microscopic venous invasion. [63, 64]

5) Alpha-L-fucosidase (AFU): It is a glycosidase enzyme found in cellular lysozomes. Although its activity can be detected in healthy individuals, increased activity is found in patients with HCC. The sensitivity and specificity of AFU for diagnosing HCC were 81.5% and 85.4% respectively. [65] In an Egyptian study, compared with AFP, AFU displayed a higher sensitivity (81.8% vs. 68.2%) but a lower specificity (55% vs. 75%). In this Egyptian study, the combined use of AFP and AFU improved HCC detection from 68.2% to 88.6%. [66]

6) Hepatocyte growth factor (HGF): It is a pleiotropic polypeptide growth factor with a number of biological activities. Its serum level was significantly elevated in HCC patients compared to patients with cirrhosis or chronic hepatitis. [67] All patients with a serum HGF concentration of greater than 0.6 ng/mL had HCC, irrespective of the AFP or PIVKA II levels. Also, HGF has been used as a prognostic marker in HCC patients, where serum HGF levels equal to or greater than 1.0 ng/ml have been associated with poor survival in HCC patients. [68]

Treatment modalities:

- Surgical resection: It is the treatment of choice for HCC in non-cirrhotic patients because of the fact that the residual liver has well-preserved hepatic reserve. [69] Patients with HCC and concomitant cirrhosis are not suitable for resection because of the potential for hepatic decompensation after surgical resection. [70]

- Liver transplantation: It is the first treatment of choice for patients with small multinodular tumors (≤3 nodules each ≤3 cm) or those with single tumors ≤5 cm and advanced liver dysfunction. The major drawback of liver transplantation is the scarcity of donors. [71]

- Local ablation: It is considered the first line treatment option for patients at early stages not suitable for surgical therapies. [72] Thermal ablative therapies emerged, and are classified as either hyperthermic treatments (heating of tissue at 60–100 C^o) including RFA, microwave ablation, and laser ablation or cryoablation (freezing of tissue at -20 C^0 and -60 C^0). [72- 73]

RFA has been the most widely used modality for local ablation of HCC. The energy generated by RF ablation induces coagulative necrosis of the tumor producing a safety ring in the peritumoral tissue, which might eliminate small undetected satellites. Consistent with previous studies, RFA requires fewer treatment sessions to achieve comparable anti-tumoral effects. [74- 78]

The technique used is percutaneous ethanol injection (PEI), which induces coagulative necrosis of the lesion. [79] It is a well-established technique for the treatment of nodular-type HCC that achieves complete necrosis in 90% of tumors <2 cm, 70% in those of 2–3 cm and 50% in those between 3 and 5 cm. [72, 73] The major limitation of PEI is the high local recurrence rate, which may reach 43% in lesions exceeding 3 cm. [80]

- Transarterial chemoembolization (TACE): TACE may offer palliative benefits for patients with intermediate stage HCC. The basis of embolization is to induce ischemic tumor necrosis via acute arterial occlusion. Embolization may be done alone (transarterial embolization) or combined with selective intraarterial chemotherapy (TACE) such as doxorubicin, mitomycin, or cisplatin and a contrast agent, lipiodol. [81]

- Adjuvant treatments to prevent recurrence: Several strategies to prevent and treat recurrence have been tested in the setting of randomized studies. Interferon is the most frequently evaluated drug so far. Different analyses have evaluated the effect of adjuvant interferon treatment. [82- 84]

- Treatments under investigation: Non-chemical non-thermal ablation techniques are currently undergoing clinical investigation. Irreversible electroporation is currently in clinical evaluation. [85] Over the last 5 years, molecularly targeted therapy has become a mainstay in the treatment of advanced HCC. Sorafenib (Nexavar) was the first and remains the only approved systemic therapy for advanced HCC. [86]

Clusterin:

Clusterin (CLU), also designated as apolipoprotein J (APOJ), sulfated glycoprotein 2(SGP2), and testosterone-repressed prostate message 2 (TRPM2). It is widely distributed in different tissues and highly conserved in species. There are two isoforms (1 and 2) with antagonistic actions regarding apoptosis. Clusterin is implicated in a number of biological processes, including lipid transport, cell adhesion, programmed cell death, and complement cascade, representing a truely multifunctional protein. [87]

CLU expression:

CLU transcript is relatively over expressed in the brain, ovary, testis, and liver, and less abundantly in the heart, spleen, lung, and breast. It was soon suggested as a marker of cell death, as it is upregulated in many cell types following cytotoxic stimulation. However, accumulating results have revealed that CLU is a much more complicated protein than initially thought. [88]

CLU gene locus and protein isoforms:

The translation product of the CLU gene in humans is encoded by an mRNA transcribed from a single copy gene, located at chromosome 8. [89] The CLU gene is expressed in almost all tissues during both development and in adults, and encodes for a glycosylated secretory heterodimeric protein of approximately 75 kDa secretory clusterin (sCLU). It has been reported that apoptotic signals in human and rodent cells can induce the production of various CLU protein isoforms, most of which remain uncharacterized.[90] Of these nonconventional isoforms, the most well-described variant refers to a stress-inducible nonglycosylated nucleocytosolic protein isoform of approximately 49 kDa (nCLU) that is encoded by an alternatively spliced mRNA of the CLU gene locus and exerts a cytostatic and proapoptotic function. [91] The CLU gene promoter is highly conserved and contains several regulatory elements that may regulate the complex tissue-specific control of the gene. Interestingly, the CLU gene is also regulated epigenetically as its promoter contains a CpG-rich methylation domain. [89]

Description:

CLU gene is 17876 bp long and contains 10 exons in total. First two exons are alternative (designated 1 and 1') used by two different transcript isoforms. Other exons (2-10) are shared with both isoforms. Several SNPs have also been found in CLU-gene, both at coding regions and at untranslated regions and introns. [92]

Transcription:

CLU gene is transcribed into 2 mRNA isoforms (NM-001831, 2859 bp; and NM-203339, 2979 bp). They result from the use of alternative first exons (1 and 1') and shared exons 2 to10. [92]

Description of protein:

Secreted CLU is produced from the transcript isoform 2. The initial protein precursor, presecretory psCLU (~60 kDa), becomes heavily glycosylated and cleaved in the ER, and the resulting alpha and beta peptide chains are held together by 5 disulfide bonds in the mature secreted heterodimer protein form, sCLU (~75-80 kDa). Also, the nuclear clusterin is first translated as a non-glycosylated protein precursor, pnCLU (~49 kDa), that is then translocated into nucleus. CLU proteins have never been crystallized, so the suggested protein structures are based on computational modeling. [92]

Localization:

The different CLU protein isoforms localize to different cellular compartments. The nuclear CLU translocated to nucleus after translation and glycosylation. The secreted CLU is initially targeted to endoplasmic reticulum, and the glycosylated protein is eventually secreted. There is also evidence of stress-caused retention of sCLU in the cytosol instead of secretion. [93]

Function of clusterin:

Clusterin was found to have many functions including: controlling cell to cell interactions, regulating apoptosis, complement, transporting lipids and acting as a (membrane policeman) protecting cells from stresses. [94] CLU exerts a small heat shock protein-like stress-induced activity. Also it has been functionally implicated in numerous physiological processes as well as in ageing and most age-related diseases including tumorigenesis, neurodegeneration, and cardiovascular and metabolic syndromes. Due to its reported distribution in both extra- and most likely, intracellular compartments, CLU has emerged as a unique regulator of cellular proteostasis. [95]

Role of clusterin in tumorigenesis:

The function of CLU in cancer genetics is controversial as regards the role of CLU in human cancers. Some investigators believe CLU to be an oncogene, others - an inhibitor of tumorigenesis. However, owing to the recent efforts of several investigators, the role of CLU in important cellular processes like proliferation, apoptosis, differentiation and transformation is beginning to emerge. [95]

The role of CLU in cancer has been the matter of debate for many years. There are many reports, mainly based on studies with cancer cell lines, indicating that CLU is involved in promotion of tumorigenesis and in conferring resistance to chemotherapeutic drugs. [96- 99] However, other studies have established that an important function of CLU is to restrict tumor development. [100] While some of the contrasting results observed so far could be explained by the use of different types of cell lines, reagents or procedures, so CLU is suggested to be lying at the crossroad of life and death and is at the same time an oncogene and a tumor suppressor gene. [95]

CLU is a prototypical multifunctional gene, it was found to regulate apoptosis; cell-cell interaction; protein stability; cell signalling; proliferation and transformation. Since CLU expression in mammalian cells is highly modulated by certain pathological processes or exposure to physical and chemical agents, CLU is mainly required to respond to exogenous or endogenous stress signals. [95, 96] In cancer, expression of CLU has been shown to be either up- or down-regulated. It is still unclear whether the opposing observations published in the literature are caused by technical reasons as the (use of different antibodies, cell lines, patients) or they reflect the fact that CLU can be a tumor suppressor and promoter, at the same time, depending on the specific biology of the disease and its phase of progression. [95]

Mechanism of sCLU action in malignancy: [101- 105]

Depletion of sCLU triggers two types of action: 1) p53 dependent; and 2) p53 independent. sCLU depletion activates p53 and alters the ratio of proapoptotic to antiapoptotic Bcl-2 protein family members, triggering mitochondrial dysfunction and apoptosis. However, Bcl-2 suppression is a p53-independent effect, such that knockdown of p53 only minimally suppressed cell death, but a pan-caspase inhibitor completely avoided cell death. Another mechanism involved in sCLU prosurvival activity is the upregulation of the phosphatidylinositol 3- kinase (PI3K)/protein kinase B (PKB or AKT) pathway and insulin-like growth factor (IGF)-1 activates the PI3K/AKT pathway through upregulation of sCLU. **Figure (1)** [106]

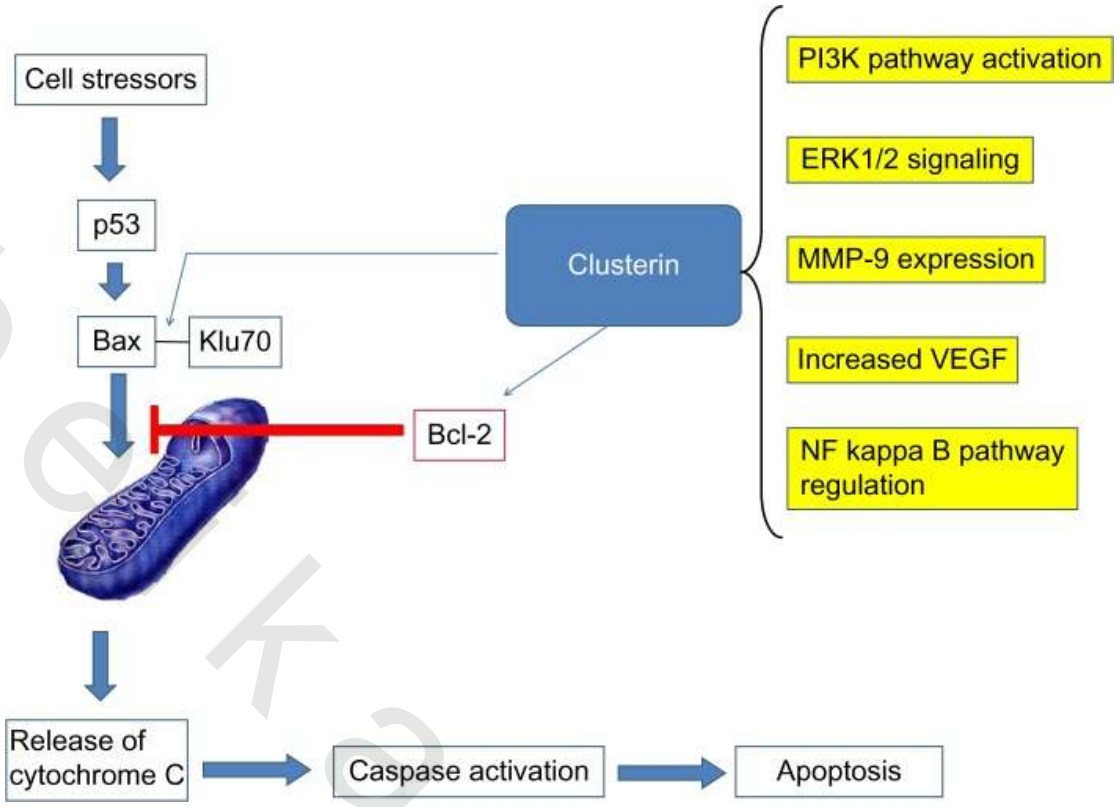

Figure (1): Mechanisms of CLU action. [106]

Implication of CLU in various diseases:

Prostate cancer: Several studies have shown decreased CLU levels in prostate cancer.[107, 108] On the other hand, there are also reports on increased expression of CLU in prostate cancer, specifically after androgen ablation therapy. [109]

Breast cancer: sCLU overexpression was associated with resistance to preoperative neoadjuvant chemotherapy in primary breast cancer. [110] Another study reported that CLU expression was associated with estrogen and progesterone receptor-negative status and with the progression from primary carcinoma to metastatic carcinoma in lymph nodes. The study also described correlation between CLU expression and tumor size. [111]

Ovarian cancer: Cytoplasmic CLU expression has been shown to increase in ovarian cancer in stage-specific manner and in response to chemotherapy as a cell-survival promoter. [112, 113] Increased CLU expression is correlated with more aggressive biologic behavior and impaired survival in ovarian cancer. [114]

Colorectal cancer: Increased cytoplasmic CLU expression has also been found in colorectal cancers. [115] CLU has also been suggested to be a potential stool biomarker for colon cancer screening. [116]

Nephrotic syndrome: CLU expression is decreased in glomerular diseases causing nephrotic syndrome, with hypercholesterolemia appearing as the unifying feature.[117]

Lung cancer: Yan Y et al [118] suggested that sCLU plays a positive role in non-small-cell lung cancer (NSCLC) cell proliferation. Also, Chou TY et al [119] found that CLU regulates epithelial-to-mesenchymal transitions and aggressive behavior of human lung adenocarcinoma cells.

Pancreatic cancer: In pancreatic cancer the reports of CLU expression are controversial, with both high and low expression. Also, it was reported that CLU expression was associated with longer survival, which supports the idea of CLU down-regulation in tumor progression. [120, 121]

Melanoma: Hoeller C et al [122] reported that, CLU expression was low in nevi but high in primary melanoma and melanoma metastases. CLU overexpression was associated with an increase in drug resistance. Shannan B et al [123] studied the expression of CLU in primary cutaneous malignant melanomas, metastasis of melanomas, and acquired melanocytic nevi. CLU immunoreactivity was found in the two former cases in contrast to acquired nevi. Treatment with vitamin D was observed to modulate CLU expression in vitamin-D responsive cells but not in resistant melanoma cell lines.

Head and neck squamous cell carcinoma: Overexpression of CLU in head and neck squamous cell carcinoma has been reported, but no relationship implications were made.[124]

Alzheimer‘s disease: Increased CLU levels are shown in Alzheimer's disease, mostly in astrocytes. Furthermore, CLU can function antiapoptotically through Bax-interference and potentiate survival mediated through TGF-beta signaling. [125]

Hepatocellularcarcinoma (HCC): High levels of sCLU expression have been associated with migration, invasion, and metastasis of HCC. It has been shown that CLU is overexpressed in metastatic human HCC tissue compared to nonmetastatic HCC tissue, implicating a role for CLU in HCC progression. [126] Recent studies showed that downregulation of CLU sensitizes cells to chemotherapy and radiotherapy. [127, 128] In HCC, it was not clear whether CLU silencing inhibited the invasion and metastasis. However, Chen D et al [129] provided evidence that CLU plays an important role in HCC invasiveness by increasing MMP-2 expression and decreasing E-cadherin expression**.** Lau SH et al [126] showed that overexpression of CLU in hepatoma cells transfected with CLU genes increased cell migration by two fold in vitro and formation of metastatic tumor nodules in the liver by eight fold in vivo.

Recent data indicate that progression towards high-grade and metastatic carcinoma leads to elevated sCLU levels and altered intracellular distribution of nCLU. Thus, the function of CLU in tumors may be related to a pattern shift in its isoform production. [130]

CLU as a therapeutic target in malignancies:

Recent findings support the concept that silencing sCLU expression can enhance the cytotoxicity of various chemotherapeutic agents, [131, 132] and demonstrate that drugs targeting sCLU expression, including CLU silencing using antisense oligonucleotides (ASO) or short interfering double-stranded RNA, may become promising tools for cancer therapy. [133, 134] Also, it has recently been shown that sCLU knockdown in human cancer

cells, using siRNA-mediated CLU gene silencing, induces significant reduction of cellular growth and higher rates of spontaneous endogenous apoptosis. [135]

Association of sCLU with multi-drug resistance, resistance to irradiation and oxidative stress:

It became clear then that CLU could mediate multidrug resistance to a broad range of unrelated chemotherapeutic agents. It can thus be concluded that multidrug resistance to a wide array of therapeutic agents used for management of cancer can be achieved by upregulation of sCLU in tumor cells. Also, it has become apparent that CLU may also be protective against radiation therapy or oxidative stress. [136]