4. INTRODUCTION

4.1 Colorectal cancer

Colorectal cancer (CRC) is one of the most common forms of human malignancy especially in industrialized countries, and is the second largest cause of cancer deaths [2]. Colorectal tumors provide an excellent system to study genetic alterations involved in the development of a common human neoplasm. Two main genetic pathways leading to CRC have been described. The first pathway, observed in 60-70% of sporadic CRCs, involves the inactivating mutations in the *Adenomatous Polyposis Coli* (*APC*) tumor-suppressor gene, the activating mutations in the oncogene *K-ras*, and inactivating mutations of *p53* tumor-suppressor gene [3]. The second pathway, characterizing the remaining 15-20% of sporadic CRCs, involves defective mismatch repair system (MMR) [1, 4]. Loss of this repair system leads to genomic instability and accumulation of a large number of mutations that in turn lead to loss of function in tumor-suppressor genes (e.g., *Bax*) and to gain of function in oncogenes (as in the case of *β-catenin*) [1].

4.2 Cellular responses to chemotherapeutic agents: Apoptosis, cell cycle arrest, and mitotic catastrophe

Chemotherapy for the treatment of cancer was introduced into the clinic more than fifty years ago. Its efficacy for the treatment of common epithelial tumors of the breast, colon, and lung is still low. Ideally, chemotherapeutic drugs should specifically target only neoplastic cells and should inhibit tumor growth by inducing cytotoxic and/or cytostatic effects without affecting normal cells. In reality, the effectiveness of chemotherapy suffers from a range of confounding factors including systemic toxicity due to a lack of specificity, rapid drug metabolism, and both intrinsic and acquired drug resistance.

Chemotherapeutic agents induce cellular responses that affect survival and proliferation of tumor cells. One of these cellular responses is apoptosis, a physiological cell death program that controls tissue growth and regeneration during development and disease

[5-8]. A second response is cell cycle arrest that allows cells to repair the damaged DNA. Mitotic catastrophe is a third kind of response to chemotherapy, during which the inability to maintain a cell cycle arrest leads cells to a premature mitosis and consequent apoptosis. The detailed molecular mechanisms of these different responses will be considered in the following paragraphs, given their importance in determining the cytotoxic action of most chemotherapeutic agents and in conferring the intrinsic resistance of some tumors to chemotherapy.

4.2.1 The intrinsic apoptotic pathway

Programmed cell death, or apoptosis, is defined by distinct morphological and biochemical changes mediated by a family of cysteine aspartyl proteases (caspases) which are expressed as inactive precursors and are proteolytically processed to an active state following an apoptotic stimulus. Two distinct signaling pathways, one external and mediated by receptors located in the plasma membrane and the other intrinsic and triggered by leakage of cytochrome c from mitochondria, lead to activation of the effectors caspases. Stress such as DNA damage can activate the intrinsic apoptotic pathway. The downstream regulation of the Bcl-2 (B-cell lymphoma/leukaemia 2) family of proteins leads to change in mitochondrial membrane permeability by directly forming pores in the outer membrane and/or by regulating the opening and closing of the permeability transition pores [9]. Members of the Bcl-2 family share some structural homology but act either to promote or inhibit cell death. Anti-apoptotic homologues include Bcl-2 and Bcl-X$_L$, and pro-apoptotic members include Bax, Bak, and Bad [10, 11]. Since these opposing family members can heterodimerize, it appears that the relative ratio of the anti-apoptotic *versus* pro-apoptotic members may determine whether mitochondria will become leaky and the cells undergo apoptosis or not. The net effect is the early and regulated release of pro-apoptotic factors from the mitochondria, e.g. cytochrome c, activation of the apoptotic protease activating factor (Apaf-1), activation of caspase-9, and subsequent cleavage and activation of downstream effectors caspases (caspase-3, -6 and -7) that finally degrade cellular proteins such as PARP and lamin [9, 12]. This gives rise to internucleosomal DNA degradation and characteristic morphological changes.

Deregulated levels of members of the Bcl-2 family of proteins are frequently associated with resistance to chemotherapeutic agents. For example, overexpression of Bcl-2 has been implicated in resistance to a wide range of anticancer drugs [13-15]; transfection of cells with Bcl-X_L causes their resistance to apoptosis induced by camptothecin (CPT), etoposide, vinblastine, paclitaxel, and cisplatin [16]; an increase in the cellular level of Bcl-X_L can prevent or delay apoptosis induced by paclitaxel [16] and many other drugs [16, 17]. Conversely, increased levels of pro-apoptotic members of the Bcl-2 family, like Bak, lead to induction of apoptosis after treatment with chemotherapeutic agents, e.g. paclitaxel [18], and overexpression of Bax protein leads to increased sensitivity to various chemotherapeutic agents, among them SN-38 [19].

4.2.2 Cell cycle arrest

An alternative cellular response to DNA damage is cell cycle arrest. A failure to arrest cell cycle at checkpoints, defined as mechanisms keeping the proper order and timing of cell cycle events, may allow the cells to enter the DNA replication process without repairing DNA damage [20, 21]. Most tumors are completely or partially deficient in at least some cell cycle checkpoints including G1/S-, G2/M-, and mitotic spindle-checkpoints. For example, more than 90% of human neoplasias have abnormality in some component of the Rb pathway: Hyperactivation of cdk2 (cyclin dependent kinase 2) as a result of amplification/overexpression of positive cofactors (e.g. cyclin D1) or downregulation of negative factors (e.g. cyclin dependent kinase inhibitors, CKIs), or mutation in the *Rb* gene [22].

The entry into the G1-phase of the cell cycle is regulated by growth factors and cell adhesion to the extracellular matrix. Extracellular signal is transmitted from cell surface receptor to nuclear transcription factors by the mitogen-activated protein (MAP) kinase cascade and in particular by the extracellular signal-regulated kinase (ERK or MAP kinase or MAPK) family of proteins. Binding of growth factors like EGF to the corresponding receptors leads to activation of the Ser/Thr protein kinase Ras; Ras interacts with and activates the Ser/Thr protein kinase Raf that phosphorylates and activates MEK1 and MEK2 (MEK or MAPK kinase or MKK). Activated MEK1/2 kinases then activate ERK1 and ERK2 proteins by phosphorylation on both a Tyr and a

Thr residue. Activation of the ERK1/2 proteins is linked to the induction of cyclin D1 mRNA [23]. D-type cyclin proteins bind and activate cdk4 and cdk6 kinases [22]. The kinase complexes cdk4/cyclin D1 and cdk6/cyclin D1 drive the cells to pass through G1-phase by two independent mechanisms. First, cdk4/cyclin D1 and cdk6/cyclin D1 complexes phosphorylate the Rb protein. The hyperphosphorylated Rb is unable to bind and to inactivate the transcription factor E2F1. Second, cdk4/cyclin D1 and cdk6/cyclin D1 complexes bind p21^{CIP1} and p27^{KIP1}, members of the family of cyclin-dependent kinase inhibitors, preventing them from binding and inactivating cdk2/cyclin E complex [24]. Because of these two effects, cdk2 kinase in combination with cyclin E (whose expression is also under the control of the E2F1 transcription factor) is strongly activated. The activity of cdk2/cyclin E complex is rate limiting for progression from G1- to S-phase, in part contributing in a feedback mechanism to the full phosphorylation of Rb protein and liberation of E2F1 molecule.

The G1/S checkpoint functions to ensure that cells do not start DNA synthesis unless sufficient nutrients are available and DNA is intact. This checkpoint relies on the inactivation of the cdk2/cyclin E complex [21, 25]. When nutrients are not available or upon DNA damage, two main mechanisms have been described to account for the inhibition of the activity of cdk2/cyclin E. Initially, p21^{CIP1} and p27^{KIP1} are rapidly released from the cdk4/cyclin D1 and cdk6/cyclin D1 complexes due to degradation of cyclin D1 through the ubiquitin-proteasome pathway. A long-term induction of p21^{CIP1} by activation of the p53 protein and an accumulation of p27^{KIP1} by unknown mechanisms are responsible for the maintenance of the G1-phase arrest [24] (**Fig.1**).
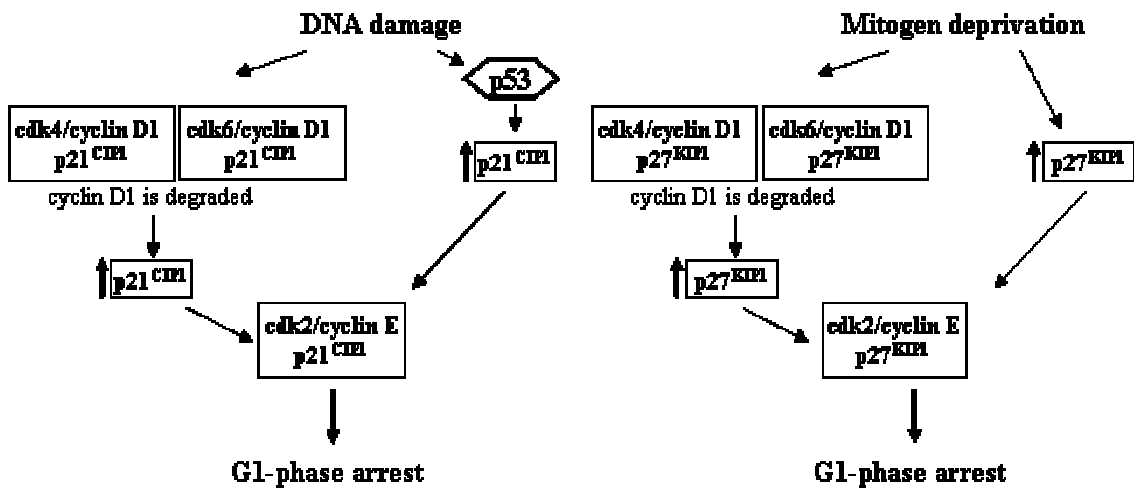
Fig. 1: G1-phase arrest in response to DNA damage or mitogen deprivation. Left; G1-phase arrest after DNA damage depends on two processes: A rapid release of p21^{CIP1} from cdk4/cyclin D1 and cdk6/cyclin D1 complexes caused by degradation of cyclin D1, and a long-term accumulation of p21^{CIP1} caused by activation of p53. Right; arrest in G1-phase in response to mitogen deprivation depends on accumulation of p27^{KIP1} due to combined inhibition of its destruction and its redistribution from cdk4/cyclin D1 and cdk6/cyclin D1 complexes to cdk2/cyclin E complexes caused by reduced levels of D-type cyclins [24].

The replication checkpoint acting in the G2-phase prevents cells from entering mitosis if there are any replication errors or DNA damage [26].

Upon termination of DNA replication, the complex cdc2/cyclin B1 and, to a lesser extent, cdc2/cyclin A regulate progression into the G2- and M-phases of the cell cycle [27, 28]. The synthesis of cyclin B1 begins at the end of the S-phase and represents the first step for initiation of the G2/M-phase progression [29]. After association of cyclin B1 with cdc2, the complex is regulated by phosphorylation. Phosphorylation of cdc2 by CAK kinase on Thr-161 is required for activation of the complex [29]. During G2-phase, cdc2/cyclin B1 is held in an inactive state by phosphorylation of cdc2 on Thr-14 and Tyr-15 by Wee1 and Myt1 kinase proteins [30-33]. Entry into mitosis is due to the activation of cdc2 through dephosphorylation on both Thr-14 and Tyr-15 by the dual specificity phosphatase cdc25C [34, 35], whose activity is counteracting the activity of Wee1/Myt1.

In response to DNA damage, the ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia related) kinases are activated, and their downstream targets chk1 and chk2 kinases are phosphorylated and activated [36-38] (**Fig. 2**). Activation of both chk1

and chk2 subsequently inactivates cdc25C through phosphorylation on Ser-216 [39, 40]. cdc25C phosphorylation creates a binding site for 14-3-3 proteins [41, 42]. cdc25C protein bound to 14-3-3 proteins is transported into the cytoplasm and thus removed from the nucleus [42]. Therefore, cdc2 remains in the phosphorylated state in the nucleus, and cells remain arrested in G2-phase [43] (**Fig. 2**).
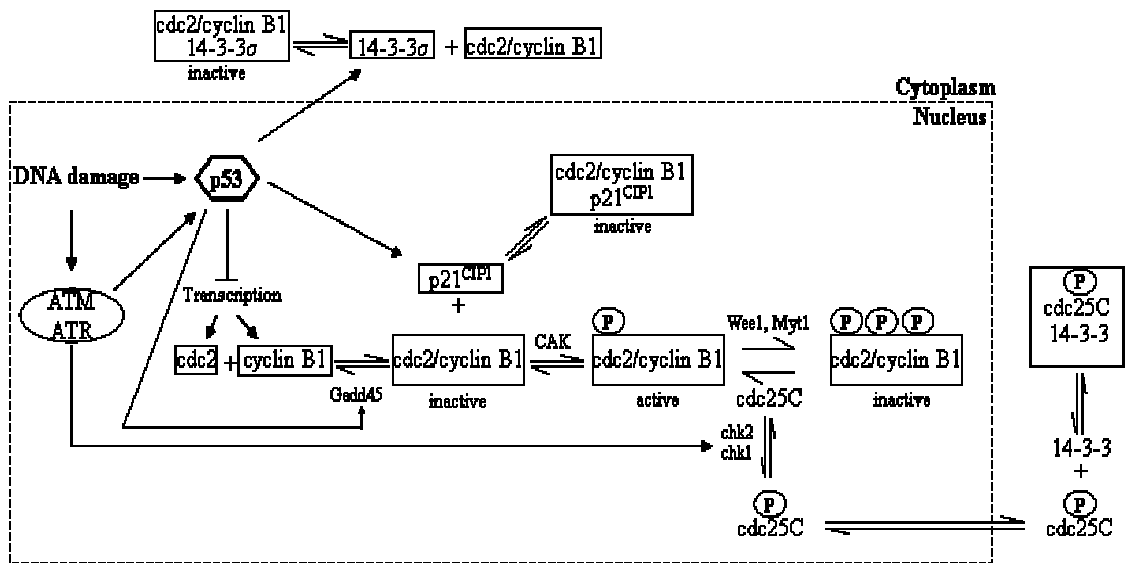

Fig. 2: Mechanisms of regulation of cdc2/cyclin B1 activity and G2/M-phase checkpoint in response to DNA damage [43].

Induction and stabilization of p53 protein after DNA damage is a second determinant regulating the G2-phase checkpoint. p53 directly transactivates *p21^{CIP1}*, *14-3-3σ*, and *Gadd45* genes [44-46]. p21^{CIP1} protein participates in the G2-phase checkpoint by inhibiting cdc2 activity through binding to cdc2 and inhibition of its kinase activity [47, 48] or by interfering with the activating phosphorylation of cdc2 by CAK [49]. 14-3-3σ is a member of the 14-3-3 family of proteins, required to move cdc2/cyclin B1 outside the nucleus thereby helping to maintain a G2-phase block [50]. Gadd45 protein is possibly involved in regulation of the dissociation of the cdc2/cyclin B1 complex (**Fig. 2**), which also contributes to inactivation of the complex [43].

4.2.3 Mitotic catastrophe

Cells with a defective checkpoint control mechanism, for example devoid of p53, p21^{CIP1}, or 14-3-3σ proteins, when insulted with genotoxic stress are not able to arrest in the G2-phase and prematurely enter mitosis leading to cell death: This process is called mitotic catastrophe [51, 52].

4.3 Role of p53 in the cellular response to chemotherapeutic agents

Exposure of cells to DNA damage and the resulting strand breaks, trigger stabilization and accumulation of the p53 protein. A key modulator of this regulatory process is the Mdm2 protein that binds to p53 and targets it for degradation by the ubiquitin-proteasome pathway. The binding between p53 and Mdm2 can be prevented by phosphorylation of p53 at sites within the Mdm2 binding region. Phosphorylation at these sites occurs in response to DNA damage and it is mediated by the kinases ATM, ATR, chk1, and chk2 [53-56] (**Fig. 3**). Since *mdm2* gene is a direct transcriptional target of p53, a negative autoregulatory feedback loop exists between these two proteins.
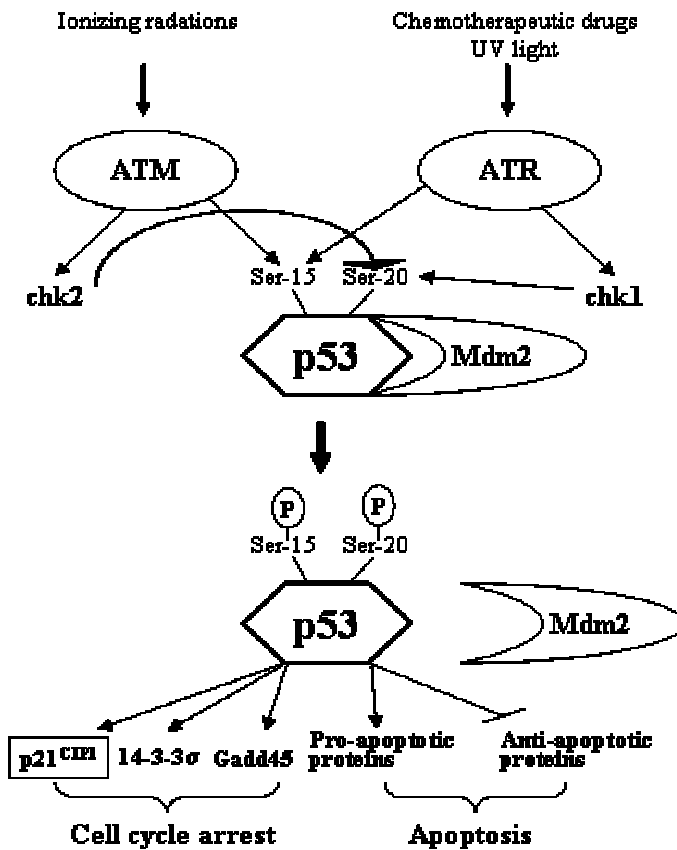
Fig. 3: After DNA damage, p53 protein is phosphorylated by ATM, ATR, chk1, and chk2 kinases. When p53 is phosphorylated, Mdm2 is not able to bind and to target p53 to the ubiquitin-proteasome degradation pathway. Accumulation of p53 results in cell cycle arrest and/or apoptosis induction [56].

Stabilization of the p53 protein because of DNA damage or cellular stress has two distinct effects (**Fig. 3**). On one side, p53 induces arrest of the cell cycle in G1- and G2/M-phases, through the transactivation of *p21^{CIP1}*, *14-3-3σ*, *Gadd45*, and, to a lesser extent, through repression of transcription of *cyclin B1* and *cdc2* genes (**Fig. 2**). On the other side, p53 induces apoptosis by activation of pro-apoptotic Bcl-2 family members (e.g. Bax, Bak, PUMA and Noxa) [57] and repression of anti-apoptotic Bcl-2 proteins: Bcl-2, Bcl-X$_L$, and IAPs (survivins) [58-61].

It is therefore clear that loss of a functional p53 protein is impairing both the apoptotic and the cell cycle responses to DNA damage. Several studies link wild-type p53 with susceptibility of the tumor cells to DNA-damaging anticancer agents [62]. This is consistent with the role of p53 as inducer of apoptosis. However, since induction of p53 leads to cell cycle arrest, it should also facilitate DNA repair and cell recovery. The inactivation of p53 can therefore enhance sensitivity to chemotherapeutic agents. This

was reported to be the case after the DNA-damaging agents paclitaxel [63], cisplatin, [64], adriamycin, and radiation [65]. The conflicting observations on p53 status and chemosensitivity suggest the possibility that different chemotherapeutic agents can have different p53-dependent effects in different tumor cells; in some settings, the p53-dependent apoptotic pathway can be activated while in others the p53-dependent cell cycle checkpoint response can be induced [66].

4.4 Role of hMLH1 in the cellular response to chemotherapeutic agents

The MMR system maintains genomic stability by repairing base-base mismatches and insertion/deletion loops originating during DNA replication, as well as heteroduplexes occurring during recombination [67]. The well-characterized MMR system in *E.coli* consists of MutS, responsible for recognition of a base mismatch, and MutL that interacts with MutS and, in the presence of ATP, activates the endonuclease MutH. This enzyme incises the newly synthesized DNA strand. Afterwards, excision and resynthesis complete the activities of the MMR system [67]. In humans, MMR system comprises proteins homologous to the *E. coli* factors MutS and MutL. hMutS, homologous of *E. coli* MutS, is a heterodimeric complex composed of hMSH2 and hMSH6 or hMSH3. The hMutSα complex (hMSH2/hMSH6) recognizes mismatches and single-base loops, whereas hMutSβ (hMSH2/hMSH3) recognizes insertion/deletion loops. Heterodimeric complexes of the MutL-like proteins hMLH1/hPMS2 (hMutLα) and hMLH1/hPMS1 (hMutLβ) interact with hMSHα/hMSHβ complexes and replication factors to drive the excision of the new strand and the resynthesis steps [67]. Although the primary function of the MMR system is to repair the damaged DNA, the MMR system has been shown to influence the response to chemotherapeutic agents by affecting the G1- and G2/M-phase checkpoints and by signaling the presence of DNA damage to the apoptotic machinery [68, 69]. Involvement of MMR system in G1-phase checkpoint has been recently demonstrated by comparing the responses of MMR-proficient and -deficient cell lines towards different DNA damaging agents [70]. The absence of a functional MMR system prevented degradation of cyclin D1 after DNA damage induced with cisplatin, mitomycin C, doxorubicin, and etoposide [70]. In

addition, after treatment with cisplatin the Rb protein was maintained in its hyperphosphorylated state in MMR-deficient cells, and in the same cell lines CKIs were not redistributed from cdk4/cyclin D1 and cdk6/cyclin D1 complexes to the cdk2/cyclin E complex. Therefore, cdk2/cyclin E kinase remained active in MMR-deficient colon carcinoma cells after DNA damage induced with cisplatin [70].

A connection between the MMR system and G2/M-phase cell cycle checkpoint has been demonstrated after treatment with mismatch-inducing agents like MNNG and 6-thioguanine (6TG) [71, 72]. hMLH1-proficient cells underwent G2/M-phase arrest while hMLH1-deficient cells escape the G2/M-phase cell cycle arrest after treatment with both MNNG and 6TG [71, 72].

As regards the role of the MMR system in induction of apoptosis, it has been observed that MMR-deficient cells are more resistant than their MMR-proficient counterparts to a wide range of chemotherapeutic drugs, in particular methylating agents, cisplatin, and 5-FU [73, 74]. The signaling pathway primed by MMR and leading to apoptosis after cisplatin-induced damage has been shown to be mediated by Abl oncoprotein and by the p53-family member p73 protein [75]. Activation of Abl kinase activity was associated with the response to cisplatin in MMR-proficient cells. MMR proficiency was also associated with stabilization of the p73 protein after cisplatin treatment, an effect that could be mimicked by cotransfection of active Abl kinase [75]. However, MMR-deficient cells showed a higher sensitivity than MMR-proficient cells to some chemotherapeutic agents, among them CCNU and mitomycin C [76]. The molecular determinants responsible for this different response were not identified.

4.5 7-Hydroxystaurosporine (UCN-01)

UCN-01 is a new antitumor agent that was isolated as a selective inhibitor of Ca^{2+} and phospholipid-dependent protein kinase C (PKC) [77]. UCN-01 has been tested both as an enhancer of cytotoxicity of other drugs and as a single cytotoxic agent.

As modulator, used at non-toxic, nanomolar concentrations, it enhances the antitumor activity of mitomycin C [78, 79], cisplatin [80-83], ionizing radiation [84, 85], camptothecin [86, 87], and 5-FU [88]. It does not enhance the antitumor activity of

other chemotherapeutic agents like adriamycin, bryostatin, bryostatin vinca alkaloids, or methotrexate [89]. The potentiation of cytotoxicity by UCN-01 is assumed to be due to its ability to abrogate the S- [87, 90] or the G2/M-phase checkpoints [85], as previously reported for caffeine [91]. The by-pass of these cellular checkpoints in the presence of DNA damage would lead the cells to death. At the molecular level, it was found that UCN-01 inhibits chk1 kinase [92-94] which leads to activation of cdc25C phosphatase and inhibition of Wee1 kinase [95], resulting in increased cdc2/cyclin B1 activity (**Fig. 4**). Thus, cells undergo mitosis followed by cell death due to the presence of damaged DNA [96].
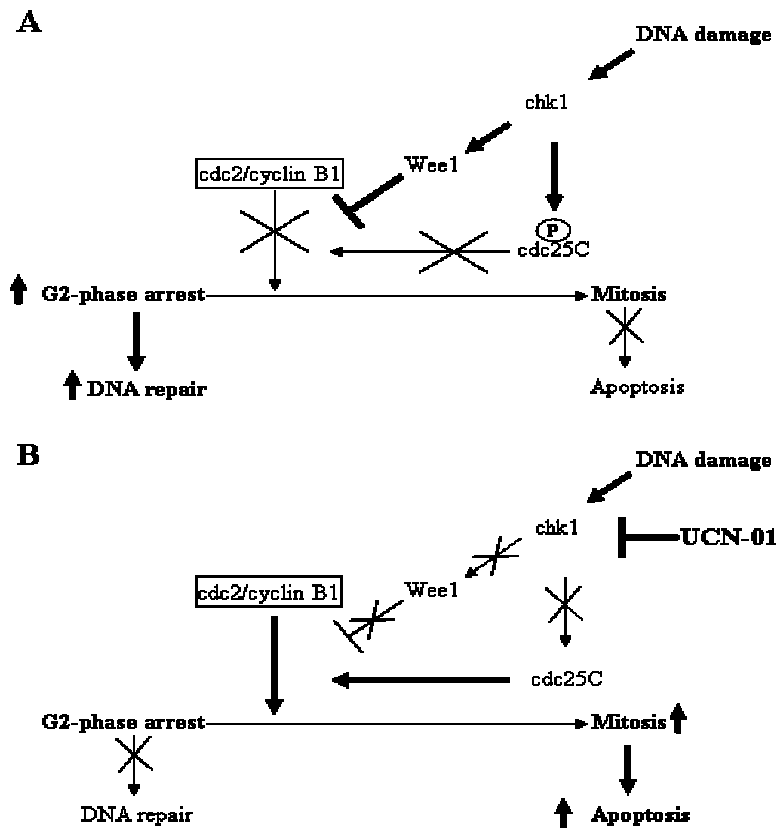

Fig. 4: Mechanism of potentiation of cyto-toxicity by UCN-01. **A**: Tumor cell lines after exposure to genotoxic stress arrest in G2-phase of the cell cycle due to loss of cdc2/cyclin B1 activity mediated by activation of chk1. **B**: Tumor cell lines after exposure to genotoxic stress in the presence of UCN-01 are unable to arrest in G2-phase and they progress to M-phase because of in-appropriate activation of cdc2/cyclin B1, leading to premature mitosis and apoptosis [94, 97].

It should be noticed for completion that in this mechanism the absence of a functional p53 protein seems to be necessary for enhancement of cytotoxicity by UCN-01. In fact, in the absence of the p53 protein, the damaged DNA will not be repaired neither by activation of the G1-phase checkpoint (mainly p53-dependent) nor by activation of the G2/M-phase checkpoint, that is abrogated by UCN-01. In the presence of the p53 protein, repair of the DNA and survival of the cells will be accomplished through activation of the G1-phase checkpoint. However, it is still debated whether p53 status is a true determinant for the potentiation of cytotoxicity by UCN-01 [97]. A group of reports described that $p53^{mut}$ cell lines were more sensitive than $p53^{wt}$ cells when exposed to different DNA damaging agents in the presence of UCN-01 [83, 85, 86, 98-100]. A single report described that $p53^{mut}$ cell lines were more resistant than $p53^{wt}$ to enhancement of cisplatin-induced cytotoxicity by UCN-01 [81]. Finally, a recent report showed that different anti-metabolites (5-FU, gemcitabine, and fludarabine) displayed a synergistic interaction with UCN-01 in a variety of cell lines independently of their p53 status [99].

At higher concentrations (0.5-1 μM) UCN-01 is exhibiting cytotoxicity as a single agent. UCN-01 induces apoptosis in various human tumor cell lines such as leukaemia [87, 101-104], colon carcinoma [87], gastric carcinoma [105], lung carcinoma [106], breast cancer [107, 108], glioblastoma [109], late stage melanoma [108], osteosarcoma [110], and pancreatic tumor cell lines [97]. *In vivo*, UCN-01 is also capable to induce apoptosis in human tumor xenografts [82, 89, 111-114]. In humans, phase I clinical trials showed activity of UCN-01 towards lymphomas [115] and, in combination with fludarabine, towards chronic lymphocytic leukaemia (CLL) [116].

The precise mechanism of UCN-01 antitumor activity is still not completely understood. UCN-01 does not interact with DNA in both cell-free and cellular systems [112] nor causes single-strand or double-strand breaks *in vitro* [117]. This suggests that UCN-01 exhibits its antitumor activity by the inhibition of PKC and/or other protein kinases. It was however clearly shown, that the induction of apoptosis by UCN-01 does not correlate with its ability to inhibit PKC activity [118, 119]. Recently, it was demonstrated in several types of epithelial cells and fibroblasts that UCN-01 inhibited activation and phosphorylation of protein kinase B (Akt), *in vitro* and *in vivo*. Inhibition

of Akt activity correlated with UCN-01 induced apoptosis, thus suggesting that UCN-01 may exhibit its cytotoxicity in part by turning off the Akt survival pathway [120].

In vitro and *in vivo*, UCN-01 selectively blocks cell cycle progression from G1- to S-phase and preferentially induces G1-phase accumulation of the target cells [78, 105, 106, 110, 111, 121-128]. UCN-01-induced G1-phase accumulation is associated with inhibition of cdk2 kinase [106, 110, 123, 124, 127]. Inhibition of cdk2 takes place by the induction of cdks inhibitors such as p21^{CIP1} and p27^{KIP1} [97, 106, 124, 127, 129] or, as it was hypothesized, by interaction of UCN-01 with the ATP binding site of cdk2 [96]. It was postulated that G1-phase arrest resulting from inhibition of cdk2 activity might lead to protection from apoptosis. Failure to inhibit cdk2 activity may be associated with apoptosis induced by UCN-01 [97, 106] (**Fig. 5**).
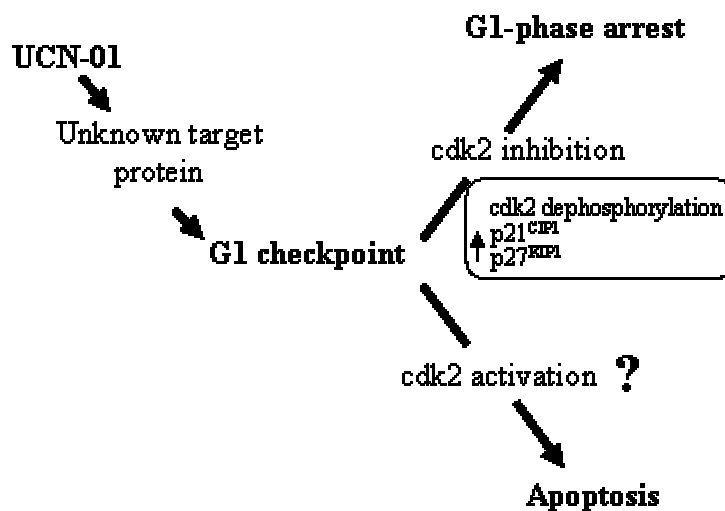

Fig. 5: Hypothetical mechanism of action of UCN-01 used as a single agent. If the cellular G1 checkpoint, controlled by an unknown target protein, is functional, UCN-01-treated cells will arrest at the G1-phase. If the cellular G1 checkpoint is not functional, UCN-01-treated cells will override the G1 checkpoint and undergo apoptosis [97].

In the present study, we wished to determine if the presence of hMLH1 or p53 proteins could influence the cellular response to UCN-01. A role of MMR system in the response to UCN-01 has never been investigated. It was previously shown that UCN-01 treatment leads to suppression of repair of cisplatin-induced DNA lesions through inhibition of the nucleotide excision repair pathway [130]. This could be a hint of some

relation between UCN-01 and the DNA repair machinery. Moreover, the MMR system is influencing the G1-phase checkpoint [70], the G2/M-phase checkpoint [71, 72], and it is probably a component of the apoptotic signaling pathway [69]. The role of the p53 protein in the response to UCN-01, used as a single agent, has already been addressed, with conflicting results probably depending on the origin of the cell lines investigated [81, 87, 102, 119, 127, 131].

4.6 Irinotecan (CPT-11)

CPT-11 is proving to be an effective complement for 5-FU, the main chemotherapeutic agent used for treatment of colon carcinoma [132]. CPT-11 induces remissions in about 11-23% of 5-FU resistant tumors [133, 134], and the combination of CPT-11 and 5-FU-leucovorin has been approved as first line chemotherapy for patients with metastatic colorectal cancer [132, 135]. The molecular mechanisms behind the therapeutic effects of CPT-11 have not yet been fully elucidated.

CPT-11 is converted *in vivo* into its active form, SN-38, by carboxylesterases that are abundantly present in the liver [136]. SN-38 exerts its cytotoxic effects by binding to and inhibiting the DNA-associated nuclear enzyme topoisomerase I (topo I). Topo I facilitates the unwinding of DNA during replication and transcription by transient nicking and subsequent religating the DNA, and SN-38 prevents topo I from rejoining the transient DNA breaks. The collision of SN-38-stabilized topo I-bridged DNA breaks, referred to as cleavable complexes, with moving replication forks leads to lasting single- and double- strand breaks and to cell death. Since the activity of topo I is frequently higher in colorectal tumors than in the normal colonic tissue, it may confer tumor specificity of the CPT-11-mediated effects [137], and a decrease of topo I activity may contribute to the resistance of tumor cells [138]. The response *in vivo* appears to correspond to the amount of cleavable complexes [139, 140], although this correlation was not found in SCLC and NSCLC cell lines [141]. CPT-11-induced apoptosis could be demonstrated in colon carcinoma cells through PARP cleavage *in vitro* and *in vivo* [142]. The mechanism of apoptosis induction is not understood [143]. Along with the increase of topo I activity, tumor-associated deficiencies in DNA repair

and in regulation of the cell cycle and/or inability of cancer cells to repress apoptosis were also suggested to additionally contribute to the cancer specificity of CPT-11. In particular, the influence of lesions in the *p53* gene and in the MMR system on the efficacy of CPT-11 have been investigated by several authors; the results, however, appear to depend on the assay system used.

Several fibroblast cell lines [144] and an ovarian A2780 cell line [145] did not show in clonogenic assays any influence of p53 status on the sensitivity to SN-38. On the other hand, the extent of apoptosis measured with TUNEL appeared to be lower in p53-defective A2780 cells [145], while in the colon carcinoma cell line HCT116 and in the breast cancer cell line MCF-7 the inactivation of p53 increased the susceptibility to camptothecin (CPT), the parental compound of CPT-11 [146]. In xenografts, a mutation in the *p53* gene was associated with resistance to CPT-11 treatment of some colon carcinoma cell lines [147] and of a human lung cancer cell line [148].

The MMR status did not appear to have a decisive effect on the sensitivity to CPT-11 of colon carcinoma xenografts [139, 147], while in a retrospective clinical study of patients with metastatic colorectal cancer a correlation was found between microsatellite instability and tumor response to CPT-11 [149]. Furthermore, some authors reported a correlation between MMR-defect with sensitivity [150] while others with resistance to topo I inhibitors [151, 152].