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Improving the selectivity of cancer treatments by interfering with cell response pathways

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Abstract

The cellular response to the stress induced by treatment with anticancer agents is a key determinant of drug activity. A pivotal role in this response is played by checkpoint proteins that control the normal passage of cells through the cell cycle. There is evidence that cancer cells often have defects in one checkpoint control that makes them more vulnerable to inhibition of a second checkpoint, thereby enhancing the overall response to treatment. The G1 and G2 checkpoints are particularly crucial for the decision of a cell to arrest in the cell cycle after damage. The checkpoints are used to try to allow the repair of any damage, or to activate the apoptotic (programmed cell death) machinery. Inhibition of both G1 and G2 checkpoints in cancer cells is therefore likely to result in an induction of the death response in cancer cells. Similarly, an increasing knowledge of the molecular mechanisms that form the basis of apoptotic pathways has helped to define why cancer cells have a reduced propensity to undergo apoptosis following the activation of apoptotic inhibitory pathways or the inhibition of pro-apoptotic pathways. Therefore, the possibility to modulate these pathways is likely to result not only in the increased activity of anticancer agents, but also in an increase in their specificity.

Keywords: Checkpoints; Cancer; Cellular response; Treatment response; Anticancer agents

1. Cellular checkpoints

When a cell undergoes an insult, mammalian cells have a well organised system that is able to work concertedly to restore, whenever possible, the normal cellular status. Depending on the type of lesions occurring at the cellular level, different sensors of damage are activated and, as a consequence, different pathways of downstream signalling and effector proteins are recruited. Such activated systems act primarily by transiently blocking cell progression through the cell cycle to enable repair enzymes to work. Once cells have been repaired the cell cycle can be restarted. However, when the lesions induced are unrepairable, the cellular response machinery activates the apoptotic pathway. This results in the elimination of cells which otherwise could pass on their genomic alterations to daughter cells.

The cell response pathway involves proteins that are able to recognise the lesion/insult and to signal to checkpoint proteins which, in turn, are able to activate the effectors. The proteins participating in this pathway are mostly protein kinases that are able to induce activating-post-translational modifications in their substrates.

Following treatment with anticancer agents, the cell cycle machinery represents the main target of the cell response pathway. For each phase of the cell cycle, there is a distinct and characterised checkpoint, which in normal cells ensures a high cellular stability throughout the entire cell cycle.

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1.1. G1 checkpoint

During the G1 phase, cells embark on critical decisions, such as the commitment to replicate DNA and to complete cell division. Therefore, the mechanism of control must be proficient. If sufficient proliferative stimuli are present, at the so-called "restriction point" [1] in late G1, a decision is made to enter into S phase. Even if cells have passed this point, genotoxic stress can still activate checkpoints which delay any further progress into S phase. At the restriction point, cells move from mitogen-dependent to mitogen-independent growth.

During the G1phase, two temporally-distinct checkpoints can be envisaged. The first is a very rapid induction of G1 arrest that is not dependent on transcription and protein synthesis [2]. Emerging evidence suggests that targeted ubiquitination of the phosphatase CDC25A, which normally abrogates the inhibitory phosphorylation of CDK2, is a rapid and efficient system to halt cell cycle progression. The signal activating the proteasome-mediated degradation of CDC25A is its phosphorylation by the proteins, chk1 and chk2 [2–4].

The second G1 checkpoint is a late G1 arrest, which is transcription-dependent. This checkpoint is mainly governed by the product of the tumour suppressor gene, *TP53*. This nuclear protein is normally present at very low levels in undamaged cells. This is because it is rapidly exported to the cytoplasm by binding with the mdm2 protein and degraded by the proteasome [5,6]. Upon damage, a very rapid accumulation of p53 is observed in the nucleus. This accumulation requires the detachment of mdm2, and is likely to be a consequence of post-translational modifications of p53, particularly phosphorylations at the N-terminus (the region that binds mdm2) [7–9]. p53 is a transcriptional factor which, upon damage, is stabilised, activated and able to transactivate a number of genes, including p21 which has a key role in determining the G1arrest. Indeed, p21 is a cyclin-dependent kinase inhibitor with a high affinity for the cdks that act during the G1/S transition.

1.2. S checkpoint

The S phase checkpoint is a transient phenomenon which delays the rate of DNA synthesis in response to DNA damage. By contrast to the G1 and G2 checkpoints, this checkpoint lacks a maintenance component that results in a delay, and not a permanent arrest of cells with incompletely duplicated genomes that have entered S phase [2,10,11].

This checkpoint is conserved from yeasts to mammalians and the proteins participating in mammalian cells include ATM (ataxia telangectasia mutated), nibrin and the recombinational repair protein, Mre11 [11–13]. These proteins have been found to be linked together and the fact that a protein, directly involved in DNA repair, is required for the S-phase checkpoint suggests that this particular checkpoint may actively regulate DNA damage repair during S phase. This would imply that the slow-down of DNA replication induced by the activation of the S-phase checkpoint not only provides time to repair the cell, but hints at the existence of an active, checkpoint-dependent association among the replication, recombination and repair pathways [11,14].

With the activation of the S-phase checkpoint, DNA synthesis proceeds in the presence of DNA damage at a reduced rate, thereby allowing the machinery to deal with any DNA lesions that might be encountered. However, a number of lesions will remain and will subsequently activate the G2 checkpoint. A permanent arrest during DNA replication would be detrimental, because it would limit the amount of template that is available for efficient repair by homologous recombination and could cause the inappropriate re-start of DNA synthesis in areas where it has already been started, with the risk of over-replication of partial genomic regions [15,16]. Recently, the chk1 protein kinase was demonstrated to participate in this checkpoint [17].

1.3. G2 checkpoint

All of the data in the literature indicates that the cyclin-dependent kinase, cdc2, plays a central role, not only in the progression from G2 phase to mitosis [18,19], but also in the activation of the DNA-damage G2 checkpoint [20,21]. Phosphorylation of the tyrosine 15 residue (and threonine 14) of cdc2 prevents the activation of the cyclin B/cdc2 complex that is needed for cells to proceed out of G2 [18,22], and is necessary and maintained during the arrest of cells in G2 after irradiation [23].

The inhibition of the cdc2/cyclin B complex is achieved in several ways. Signals can induce the activity of kinase(s) that phosphorylate the inhibitory site, tyrosine 15 (WEE1), or can induce the activation of specific phosphatases (CDC25 A, B and C) or can result in the binding and/or sequestering of the cdc2/cyclin B complex [18,20,24,25]. The dephosphorylation of residues (tyrosine 15 and threonine 14) is crucial to allow the activation of the kinase, cdc2, and it is mediated by the CDC25 dual phosphatases (CDC25A, B and C), all of which are involved in G2 checkpoint activation [20,26,27]. CDC25C can activate the complex only following its translocation to the nucleus; in fact, it is generally localised in the cytoplasm bound to 14-3-3 proteins (not the sigma isoform) [27,28]. The region of CDC25C interacting with 14-3-3 proteins contains a phosphorylation site, serine 216, which is phosphorylated throughout interphase, but not during mitosis [29]. The kinase responsible for the phosphorylation of

CDC25C on serine 216 during interphase has been cloned and named C-TAK1 (for *C*dc *T*wentyfive C *A*ssociated protein *K*inase) [30]. Such a phosphorylation event, that renders the phosphatase inactive, has been shown to occur after cellular stress and is mediated by two kinases, chk1 and chk2, although *in vivo* evidence would suggest that the major kinase responsible for this phosphorylation is chk1 [31–33]. A requirement for CDC25A-induced degradation during G2 phase checkpoint activation has been recently postulated. Again, chk1 and chk2 kinases have been involved in CDC25A phosphorylation (at serine 123), with its enhanced degradation resulting in the sustained inhibitory phosphorylation of cdk2 (leading to G1 arrest and a block in S phase entry) and cdk1 (leading to a G2 block). Finally, the CDC25B phosphatase seems to be more important in the initiation of the G2/M checkpoint, rather than in its maintenance [21]. Phosphorylation of CDC25B by the p38 mitogen activated protein kinase (MAPK) increases the ability of the phosphatase to bind to the 14-3-3 protein [34]. This mechanism has been clearly observed following ultraviolet (UV) radiation, but not with other types of damage suggesting that it could represent a "damage-specific" checkpoint activation.

Although p53 has been mainly involved in regulating the G1 checkpoint, it also has an important role in controlling the G2/M transition [35]. p53 is able to activate the transcription of three distinct genes that interfere with the activity of cdc2: the *p21* gene which inhibits cdc2 kinase activity [36], the *GADD45* gene, which is able to dissociate the complex between cdc2 and cyclin B [37,38] and the *14-3-3 sigma* gene [39]. Its product, the protein 14-3-3 sigma, inhibits cdc2 activity via a mechanism that is distinct from those activated by p21 or GADD45. In fact, the 14-3-3 sigma protein is able to bind cdc2 and to anchor the complex cdc2/cyclin B in the cytoplasm [35,40]. Prevention of the nuclear translocation of cdc2/cyclin B is sufficient to halt cells in G2. Moreover, p53 can induce G2 arrest through direct repression of cyclin B and cdc2 gene transcription [41,42].

1.4. M checkpoint

Moving towards mitosis, cells must distribute their replicated genetic material evenly between the two daughter cells. In this phase, microtubules organise into a bipolar spindle which segregates the duplicated chromosomes [43]. This is under very tight control because mis-segregation of chromatids will lead to aneuploidy [44]. In human cells, if centrosome separation (necessary for the bipolar spindle formation) does not occur, prometaphase is delayed and the mitotic checkpoint inhibits the chromatids separation until all the kinetochores are attached to the microtubules [45]. Moreover, a further control checks the exit from mitosis and is able to block this exist until completion of the chromosome separation [46,47]. The crucial steps in the progression through mitosis are controlled by the disruption of the mitotic inhibitory proteins, a phenomenon occurring when these proteins are ubiquitinated by anaphase promoting complex/cyclosome (APC/C) and targeted to the proteosome for degradation [48,49]. In order to ubiquitinate the substrates, APC/C must be complexed with the protein, cdc20 (50). The mitotic checkpoint directly inhibits APC/C function [50]. The genes involved in this checkpoint have been discovered in *S. cerevisiae* and mammalian homologues have been identified [51,52].

2. Therapeutic exploitation of the modulation of cellular checkpoints

Almost invariably, human tumours present alterations in one or more proteins involved in the cellular response pathway. These alterations can be either mutations/deletions/amplification at the DNA level, or alterations in transcription, i.e. hypermethylation of CpG islands, or alterations in protein synthesis and/or degradation. Whatever the mechanism, cancer cells are able to escape control mechanisms, giving rise to clones that present with a growth advantage over normal cells.

Can the altered pathways governing cell cycle checkpoints and the cellular response be exploited to develop new therapeutic strategies aimed at improving the activity of anticancer agents? And, more importantly, can this be used to increase the selectivity of anticancer agents? The theoretical basis for selectivity is that the block of one of the mechanisms controlling cell cycle progression should have a much greater impact in cells with an already compromised response pathway (Fig. 1).

2.1. Modulation of G1 checkpoint

Among the genes and pathways most commonly altered in human cancer, p53 represents the best example, and is probably the most frequently altered gene in humans. The lack of p53 (or the presence of a mutated protein) invariably leads to genome instability and to the accumulation of mutations in DNA. Restoration of p53 function therefore represents an attractive possibility to induce a response in those cells harbouring a mutated form of the protein, and there are already data that suggest this approach can indeed lead to a pharmacological approach. In many cancer cells, the re-introduction of a wild-type (wt)-p53 leads to growth arrest and/or apoptosis [53–55], although the presence of multiple alterations in other genes might mask or make a complete response unlikely with this kind of treatment alone.

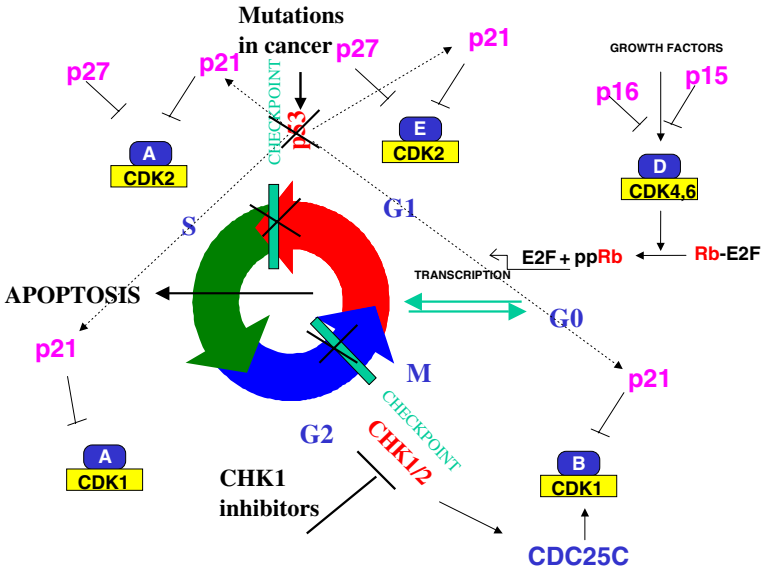

Fig. 1. Schematic representation cell cycle regulation. Checkpoints proteins and the expected induction of apoptosis in cancer cells with compromised checkpoints are underlined.

p53 has an essential role in controlling the G1 checkpoint and is an essential protein dictating the fate of a cell following damage [53,56,57]. Depending on the cell type and the kind of damage induced, p53 is able to activate a cell cycle arrest or to activate apoptotic processes. This means that cancer cells with inactivated p53 can have an attenuated apoptotic and/or cell cycle arrest response. If for a given tumour the p53-dependent apoptotic response predominates, then the lack of p53 is likely to result in a reduced response to drug-induced apoptosis. Conversely, if p53-dependent cell cycle arrest is the predominant response, it is possible that tumour cells will not arrest in G1 following treatment, will not repair the lesion and will eventually die. From a therapeutic point of view, it would be important to have drugs with increased activity against cells with inactivated p53. This theoretically will result in an increased therapeutic index, with normal cells that are often targets of toxicity (i.e. bone marrow cells) still able to respond through a wt-p53 mechanism. Different isogenic cellular systems have been generated with the aim of determining the role of p53 in the cell's response to treatment [58–64] and these systems can be used to screen new molecules with enhanced activity in p53-mutated cancer cells. The availability of these cellular systems, for virtually all cancer types, will make it possible to predict tumour chemosensitivity based on the cell's p53 status.

Furthermore, p53 undergoes different post-translational modifications (mainly phosphorylations and acetylations), and these different modifications likely dictate the kind of genes that are transcriptionally regulated [8,65–68]. In particular, the p53-dependent transcription of some pro-apoptotic genes, such as *AIP* (apoptosis inducing protein), seems to be dependent on specific phosphorylations [68], and this offers the possibility to search for molecules or treatment regimens that are able to favour the activation of the kinase(s) responsible for the specific phosphorylation and, hence, the onset of an apoptotic response.

Another interesting possibility, which already has some experimental supporting evidence, is the possibility to restore p53 function in cancer cells. This approach would have the specificity of acting in cells harbouring mutations in the *TP53* gene (i.e. cancer cells) and not, or only negligibly, in cells with an intact *TP53* gene (i.e. normal cells). The assumption is that, at least for certain mutations, it is possible to find molecules which, upon binding to the mutated protein, are able to induce a shift in the conformation of the mutated protein allowing the formation of a wt-like protein with restored DNA binding and *trans*-activation abilities. Experimental data have already been presented [69,70] that peptides able to bind to mutated p53 can indeed restore the transcriptional activity of mutated p53. These studies indicate that this strategy is feasible and are interesting, particularly considering that the restoration of p53 activity could lead, in some cancer types, to an increased cell apoptotic response following treatment to anticancer agents. Moreover, it is expected that the restoration of p53 function *per se* should already have effects on cancer cell growth.

2.2. Modulation of the G2 checkpoint: chk1 and chk2 as targets for the development of new anticancer agents

The G2 checkpoint is much less altered in cancer cells than the G1 checkpoint. Mutations in proteins like chk1

and chk2 [71,72], and downregulation of chk2, due to promoter hypermethylation, in Hodgkin's lymphoma and lung cancer [73,74] have been reported. However, most tumours have a functional G2 checkpoint and are able to arrest in G2 following treatment with damaging agents.

Inhibitors of chk1 and chk2 have been proposed as G2 abrogators, particularly in combination with anticancer agents. The rationale for the development of these agents is that damaged cells will not arrest in G2, will enter mitosis, but, if the damage is sustained, there will be a ''mitotic catastrophe''. This effect is likely to be more detrimental for those cells with an already compromised G1 checkpoint (i.e. most cancer cells), while normal cells should still be able to activate the G1 checkpoint response. The results obtained so far are encouraging, although the drugs now available do not possess a very high target selectivity, thus masking or reducing the effects. The use in pre-clinical systems of small-interfering (siRNAs) that are able to selectively block the expression of a desired protein support this hypothesis and also showed a potentiating effect when combined with anticancer agents. Studies from our laboratory have demonstrated that this effect is negligible in cells with an intact G1 checkpoint, while a potentiation of the cytotoxic activity of drugs, such as cisplatinum and ionising radiation, are observed in cells with a compromised G1 checkpoint (i.e. cells without p53) [75].

While data on chk1 are consistent, the role of chk2 in determining the cellular response to damage has been questioned by two independent observations [76,77] and by data obtained in our laboratory [75]. These studies clearly showed that cells without chk2 were still able to be blocked in G2 (possibly through chk1) and that inhibition of chk2 alone does not translate into a modification of the *in vitro* cytotoxic activity of drugs, such as DDP. We would therefore question using chk2 as a target for new anticancer agents.

Data reported on the differential effects of chk1 and chk2 abrogation on the G2 checkpoint and on the response to treatment, strongly encourages the design of molecules with the ability of selectively target chk1, but not chk2. The possibility of blocking chk1, without altering the expression and function of chk2, is likely to reduce the undesired effects arising from the unnecessary abrogation of a key protein.

3. Apoptotic response

Apoptosis, programmed cell death, has been described to be alterated in many human tumours, implying that destruction and/or impairment of the apoptotic pathway can contribute to the pathogenesis and progression of tumours. In addition, apoptosis has been shown to be important, not only for the chemotherapy- and radiotherapy-induced-killing of cancer cells, but also in playing a role in tumour chemoresistance and radioresistance, with an increased threshold for cell death that would require higher doses for tumour killing. Two major pathways of apoptosis can be recognised: the dead-receptor-induced extrinsic pathway and the mitochondria-apoptosome-mediated intrinsic pathway. Both pathways lead to caspase (intracellular cysteine proteases) activation and cleavage of specific cellular substrates [78–80]. The players of note in the extrinsic pathway are ligands and their receptors such as FAS, tumour necrosis factor (TNF), tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and downstream molecules, such caspases and Bcl2, family member proteins; while in the intrinsic pathway, the release of cytochrome c is pivotal in the switching of the apoptotic process and such release depends on the balance between pro-apoptotic and anti-apoptotic Bcl2-family proteins [81–83].

4. Therapeutic implications of the modulation of the apoptotic pathway

In recent years, a better understanding of the molecular process at the basis of apoptosis (mainly the fact that the balance between pro-apoptotic and anti-apoptotic proteins is the determinant for the switching on and off of the process) has unravelled novel strategies that can be employed for the treatment of tumours. For solid tumours, in particular, a low propensity to undergo apoptosis has been associated with a low response to treatment. Experimental data have suggested that is theoretically possible to drive cells through the apoptotic pathways (rather than into cell cycle arrest pathways) with a dramatic increase in the sensitivity of anticancer agents [84]. Interestingly, these effects have also been observed *in vivo* in mice implanted with human tumour cells that have been engineered to preferentially express p53-dependent apoptotic genes [84]. This allows alterations in the apoptotic pathways in cancer cells to be identified and exploited and possibly used in synergy with currently available anticancer drugs.

4.1. Inhibitors of apoptotic proteins

The discovery of endogenous antagonists of the anti-apoptotic Bcl2 and IAP (inhibitor of apoptosis protein) family proteins (such as XIAP, c-IAP1, c-IAP2 and survivin) able to bind directly to and inhibit different caspases [85,86], and the finding that the peptidyl motifs in their protein sequence are necessary to overcome apoptosis suppression will certainly lead to the synthesis and production of non-peptidyl small molecules antagonists to treat tumours. Very recently [87], small inhibitors of

XIAP have been described. These small molecules are able to block the XIAP-induced inhibition of caspase-3. Intriguingly, these molecules were effective in inducing apoptosis in cancer cells, with limited toxicity against normal cells. This would imply that caspase activation is different in normal and cancer cells, and that, in the latter, caspases are already activated.

The unravelling of the role of nuclear factor (NF)-κB as an important transcription factor involved in the transcriptional regulation of different anti-apoptotic genes, including *c-IAPs*, *Bcl-2* and *Bcl-X*, has made it an attractive target. NF-κB binding to different inhibitory molecules (IkBα, IkBβ, p105 and p100) sequesters, and thereby inactivates, NF-κB in the cytoplasm. NF-κB activation procedes through the degradation of its inhibitory molecules and its consequent translocation to the nucleus, where it induces transcription [88,89]. It has been reported that elevated levels of NF-κB are found in several types of human tumours [90–92]. NF-κB has been shown to be inhibited by the proteosome inhibitor, velcade (PS341), through the blocking of Ikβ degradation and by PI3-K Phosphatidylinositol 3-kinase inhibitors, through the inhibition of Ikβ phosphorylation [93–95]. Moreover, small molecules able to inhibit the activity of NF-κB-activating kinases (IKKs) could also be useful to restore the apoptotic sensitivity of cancer cells [91].

Antisense therapy, aimed at decreasing the expression of different antiapoptotic genes (*Bcl2*, *XIAP*), has also been evaluated. G3139, an antisense phosphorothioate oligodeoxynucleotide that suppresses Bcl-2 expression, is now in phase III clinical trials. Preclinical studies have shown that G3139 increases the antitumour activity of different cytotoxic agents, suggesting that its combination with conventional chemotherapy could be effective in the clinic, particularly because initial clinical studies demonstrated that G3139 is also well tolerated.

4.2. Apoptotic inducers

Several studies have tried to induce apoptosis in human cancers, interfering with both the extrinsic and intrinsic pathways. The elucidation of the mechanisms involved in the activation of the pathways have been instrumental in defining of the targets to be selected for the screening of new molecules.

The family of TRAIL proteins and receptors has attracted researchers from many laboratories, based on the observation that TRAIL preferentially induces apoptosis in cancer cells compared with normal cells. The presence of decoy receptors for TRAIL, as well as the role of intracellular proteins such as FLIP (Flice-inhibitor protein), a key factor in signal transduction pathways, accounts for the specificity of TRAIL in preferentially inducing apoptosis in cancer cells [96,97]. Moreover, data from different laboratories indicate that the treatment of cancer cells with recombinant TRAIL plus chemotherapeutic agents has an activity that is superior to that found when the two regimens are given separately. Interestingly, these results have been found in different cancer cells, both *in vitro* and *in vivo*, in animals bearing human tumours [91,98,99]. The availability of agonistic monoclonal antibodies directed against the R1 receptor, that are found in several cancer cells, can also be exploited therapeutically. However, it should be that the specificity of TRAIL for cancerous rather than normal cells has to be seen as a preferential activity, and in fact TRAIL has been reported to induce apoptosis in normal cells, such as hepatocytes as well [100]. Thus, caution should be exercised when considering the use of these treatments in humans as more experimental studies are needed.

The (PI3-K)-akt pathway is another recognised target for the design of new anticancer agents (Fig. 2). In several cancer cells, the overexpression/amplification of PI3-K or the absence of the PTEN phosphatase (which dephosphorylates PI3-K) leads to a constitutive activation of the anti-apoptotic protein, akt [13,101,102]. This activation is likely to reduce the apoptotic potential of these cancer cells and there are already data that suggests the inhibition of PI3-K activity correlates with a decreased amount of activated akt and with an enhanced apoptotic rate of cells exposed to PI3-K inhibitors and DNA damaging agents [103,104]. In particular, the use of inositolphosphates has been shown to effectively reduce the amount of phosphorylated akt and to synergise with DDP in inducing apoptosis in dif-

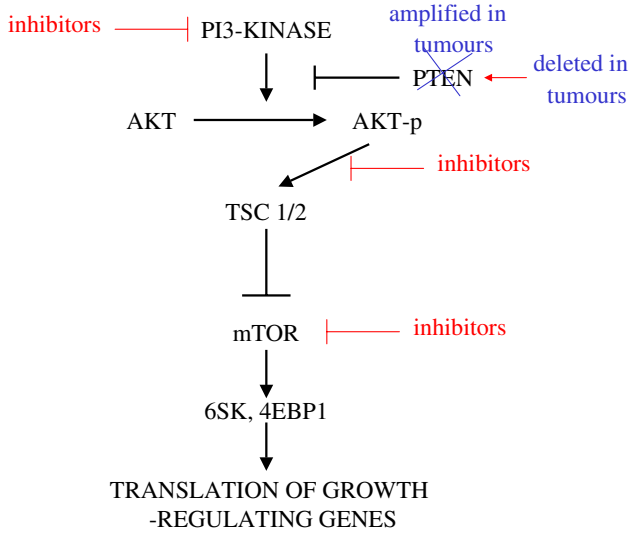

Fig. 2. Schematic representation of the Phosphatidylinosital 3-kinase (PI3-K) pathway with an indication of the target already selected for the design of molecules that are able to block the cascade and to favour drug-induced apoptosis.

ferent experimental systems [103]. The rationale for the specificity of PI3-K/akt inhibitors is the widely reported alterations of these pathways in human cancer and is not due to the presence of specifically modified protein(s) present in tumours. A careful examination of the molecular profile of human tumours is needed to assess the potential benefit(s) of these treatments.

Among the different downstream targets of akt, mTOR is gaining an increasing importance. mTOR is a serine/threonine kinase whose name is related to its natural inhibitor, rapamycin (Target Of Rapamycin). Rapamycin, was in fact the first inhibitor of this protein and is currently used as a potent inhibitor of T-cell activation [105,106]. The protein structure of mTOR has enabled the identification of different domains. Interestingly, the C-terminus of mTOR shares a high similarity with the catalytic domain of PI3-K. The mechanism by which mTOR is signalled by akt probably involves the phosphorylation of TSC-2 which is normally (in its unphosphorylated state) bound to TSC-1 in a complex that is able to inactivate mTOR. Phosphorylation of TSC-2 (by akt) leads to the dissociation of this TSC-1/TSC-2 complex and activation of mTOR kinase activity. mTOR is able to activate different substrates, including the ribosomal S6K1 and the IF4E binding proteins (4EBPs), both of which play crucial roles in regulating translation [105,106]. Moreover, the synthesis of specific proteins seems to be regulated through mTOR/S6K1 and 4EBP1, since inhibition of mTOR induces an approximate 5% inhibition of protein synthesis in cells. Cyclin D1 and c-myc are among the proteins affected by TOR inhibition, both have cap sites in their 5′ UTR. As a consequence, both cyclin D1 and c-myc levels are reduced by treatment with rapamycin.

Different new mTOR inhibitors have been described as possible new anticancer agents and some of them are currently under clinical investigation [105,107]. In clinical studies, attempts have been made to measure the blood concentrations of proteins likely to be downregulated by treatment. This is essential information when efficacy has to be correlated with the ability to downregulate the target. The specificity of action of mTOR inhibitors, as for akt-PI3-K inhibitors, is thought to mostly rely on the constitutive activation of such pathway(s) in many cancers. In addition, data indicate in cells lacking p53 (i.e. most human tumours), rapamycin is able to induce apoptosis, while the expression of wt-p53 protects these cells from rapamycin-induced apoptosis, adding a further degree of selectivity to this class of compounds. Furthermore, for this new and interesting class of molecules, their combination with conventional or new anticancer agents has proved to be synergistic or additive [108,109], indicating that combination is not only possible, but can produce benefits compared with single treatments.

5. Concluding remarks

Our increasing knowledge about the human genome, and of the complexity of interactions between genes, proteins and molecular pathways, has resulted in promising new targets for the design of new anticancer agents. These should possess high antitumour activity and a higher specificity for cancer cells and hence a higher therapeutic index than conventional anticancer agents. However, it should be noted that conventional chemotherapy is far from useless and is still a powerful tool, albeit with a low specificity, for the treatment of cancer. Together with the design of new target-oriented drugs, efforts should be made to find and test molecules that are able to increase the selectivity of already available anticancer agents, in order to improve their therapeutic indeces. The possibility of altering the function of checkpoint proteins in cancer cells follows this rationale. We obviously need a good knowledge of the molecular characteristics of human tumours in order to give a target-specific drug to a patient with a tumour that is likely to respond to such drugs. Measurable markers of effective target inhibition following treatment are also required.

Finally, human tumours are characterised, not only by interpatient heterogeneity, but also by intra-patient heterogeneity, which likely limits the effectiveness of a single target-oriented drug in eradicating the tumour. For this reason, the possibility to ''guide'' powerful, anticancer agents that have a broader substrate range towards cancer cells whilst protecting normal cells by interfering with cellular response pathways remains an attractive possibility to enhance the anticancer drug response.

Conflict of Interest statement

None declared.

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