

## Review

## Cyclin D1 and human neoplasia

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**Abstract**

**Neoplasia is characterised by abnormal regulation of the cell cycle. Cyclin D1 is a protein derived from the PRAD1, CCND1 or bcl-1 gene on chromosome 11q13, which is involved in both normal regulation of the cell cycle and neoplasia. In the G<sub>1</sub> (resting) phase of the cell cycle, cyclin D1 together with its cyclin dependent kinase (cdk) partner, is responsible for transition to the S (DNA synthesis) phase by phosphorylating the product of the retinoblastoma gene (pRB), which then releases transcription factors important in the initiation of DNA replication. Amplification of the CCND1 gene or over-expression of the cyclin D1 protein releases a cell from its normal controls and causes transformation to a malignant phenotype. Analysis of these changes provides important diagnostic information in mantle cell (and related) lymphomas, and is of prognostic value in many cancers. Knowledge of cyclin D1's role in malignancy at the various sites, provides a basis on which future treatment directed against this molecule can proceed.**

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*If way to the better there be, it exacts a full look at the worst—Thomas Hardy*

This review provides a background to neoplasia and the important role of cell cycle regulators, especially the contributions of cyclin D1, in human neoplasms. In 1952 the eminent British oncologist Sir Rupert Willis<sup>1</sup> provided what for many years has been considered the most accurate definition of neoplasia when he described a neoplasm as “an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change.” Although this definition is relevant today, it may not do justice to the critical role played by the cell cycle in carcinogenesis. Many more recent investigators examining the uncoordinated growth and proliferation in neoplasms, have implicated proteins involved in regulation of the cell cycle; so much so that neoplasia is considered by some to be the result of dysregulation of the cell cycle machinery.<sup>2</sup>

Increased expression of one such cell cycle regulator, cyclin D1, is a feature of many different primary human tumours with current diagnostic and prognostic implications. The central part played by this molecule deserves more attention if many important questions regarding neoplasia are to be answered.

**Normal cell cycle**

At the centre of the cell cycle engine are a group of protein kinases, the cyclin dependent kinases (Cdks), which move cell proliferation forward by phosphorylating specific substrates in a cell cycle dependent fashion. To become active protein kinases, a Cdk subunit must associate with a cyclin subunit to form a heterodimeric molecule. Unique complexes of Cdks and cyclins with specific activities during each phase of the cell cycle ensure progression through various cell cycle transitions.<sup>3</sup>

**Cyclins**

Eight major classes of mammalian cyclins have been isolated, although within some classes a number of subclasses exist. There are at least 11 different mammalian cyclins termed A, B1, B2, C, D1, D2, D3, E, F, G, and H.<sup>4-5</sup> The different cyclins attain peak activity during different phases of the cell cycle—for example, cyclins C, D1-3, and E reach their maximum activity during the G<sub>1</sub> phase, and apparently regulate transition from G<sub>1</sub> to S, whereas cyclins A and B1-2 are most active during the S and G<sub>2</sub> phases when they regulate transition to the mitotic phase of the cell cycle.<sup>6</sup> However, it must be noted that the fluctuations in cyclin D1 are more subtle than those characteristic of the other cyclins, and may represent redistribution during the S phase rather than enhanced activity during the G<sub>1</sub> phase.<sup>7</sup>

**Cyclin dependent kinases and their inhibitors**

The activities of the seven different Cdks (Cdk 1-7) acting in concert with their cyclin partners also vary with successive phases of the cell cycle. Cdk1, also known as cell division control molecule 2 (cdc2) bound to cyclin B, has maximal activity at the G<sub>2</sub>/M transition. Cdk2 is important before and during the S phase, binding to cyclins E and A. Cdk4 and cdk6 preferably associate with the D type cyclins during the G<sub>1</sub> phase.<sup>6</sup> Also involved in cell cycle regulation is a recently discovered group

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of molecules, the cyclin dependent kinase inhibitors (CKIs).<sup>8</sup> The CKIs fall into two classes—the Kip/Cip family comprising three structurally related proteins (p21, p27, and p57), and the INK4 proteins comprising four similar molecules (p15, p16, p18, and p19). In general, the amount of CKI present provides a threshold that the catalytically operative cyclin–Cdk must overcome to drive the cell cycle forward. However, the two classes of CKI appear to exert their inhibiting effects in different ways. The Kip/Cip family are capable of binding to and inhibiting most cyclin–Cdk complexes, whereas the INK4 molecules seem to be specific inhibitors of complexes containing cyclin D. It appears that the INK4 molecules compete with D-type cyclins for binding to their Cdk partners.<sup>3</sup>

#### Cell cycle in neoplasia

Predictably, proteins involved in driving the cell cycle, such as cyclins, are frequently overexpressed in primary tumours, whereas proteins that slow cell division, such as the CKIs, are often inactivated.<sup>9</sup> Of the many cell cycle regulators implicated in the development of cancers, cyclin D1 is among the most prevalent. Overexpression of D-type cyclins has been shown to contract the G<sub>1</sub> phase, decrease cell size, and reduce the dependency of the cell on mitogens<sup>10–12</sup> in animal models and cell lines.

#### A link between D-type cyclins and the retinoblastoma protein

The connection between D-type cyclins and tumorigenesis is bolstered further by compelling evidence that D-type cyclins are important in cell cycle regulation of the retinoblastoma tumour suppressor protein (pRB), an approximately 105 kDa nuclear phosphoprotein.<sup>13–15</sup> The amount of pRB is not altered with progression of the cell cycle, however, the phosphorylation state of pRB is cell cycle dependent.<sup>16–18</sup> pRB is hypophosphorylated throughout G<sub>1</sub> phase, phosphorylated just before S phase, and remains phosphorylated until late mitosis. Hypophosphorylated pRB arrests cells in G<sub>1</sub>,<sup>19</sup> an effect most likely mediated through complex formation with DNA binding proteins (including members of the E2F family)<sup>20–22</sup> required for transcriptional activation of cellular genes. Phosphorylation of pRB during late G<sub>1</sub> phase reverses the growth suppressive effects of pRB, by untethering E2F from its inhibitory constraint and thereby allowing the activation of genes required for DNA replication.<sup>23</sup> Because D-type cyclins are able to bind to pRB through an N-terminal LXCXE motif,<sup>24–26</sup> they are excellent candidates for G<sub>1</sub> phase pRB protein kinases as part of a complex with their specific Cdk partners. Interestingly, this LXCXE motif is common to the SV40 T antigen, adenovirus E1A, and human papillomavirus E7 proteins,<sup>23</sup> which may also bind to pRB and release E2F; a fact that in part explains the oncogenic potential of these viruses. Support for the idea that D-type cyclins can inactivate pRB comes from reports that increased amounts of D-type cyclins can reverse the pRB induced cell cycle arrest and

accelerate progression through G<sub>1</sub>.<sup>12 25 27 28</sup> In some mouse lymphoma cells overexpressing D cyclins, pRB is hyperphosphorylated compared with pRB in cells not overexpressing D cyclins.<sup>2</sup>

Cells that lack a functional pRB have significantly lower amounts of cyclin D1 and cyclin D1–CDK4 complexes,<sup>29–31</sup> a result which has been interpreted to mean that hypophosphorylated pRB is involved in the stimulation of cyclin D1 transcription. The reported ability of exogenously expressed pRB to induce cyclin D1 is in accordance with this hypothesis.<sup>31</sup> Thus, a negative feedback loop seems to exist in which cyclin D1 synthesis and activation lead to pRB phosphorylation, which in turn causes decreased cyclin D1 expression.<sup>32</sup>

#### Cyclin D1 gene as the bcl-1 oncogene?

The cyclin D1 gene (CCND1), linked closely to the bcl-1 gene on chromosome 11q13, is sometimes referred to as the PRAD1 gene because of an initial finding of frequent rearrangement of this gene in benign parathyroid adenomas.<sup>33</sup> The important role of cyclin D1 in parathyroid neoplasia has subsequently been confirmed.<sup>34</sup> The bcl-1 locus, so called because of the involvement of this chromosomal region in translocations {(t11; 14) (q13, q32)} characteristic of certain B cell lymphomas (now called mantle cell lymphomas), was originally thought to be identical to the cyclin D1/PRAD1 gene, but it has been shown to reside 110–130 kb upstream or centromeric to the PRAD1 gene.<sup>35</sup> However, no transcriptional units have been identified in the immediate vicinity of the major translocation cluster (MTC)<sup>36</sup> of the bcl-1 breakpoint area or any of the breakpoints that have been found up to 63 kb telomeric to the MTC region.<sup>37</sup> The absence of CpG islands between the original bcl-1 locus and PRAD1's CpG island<sup>35 36 38</sup> lends further support to the notion that no other gene lies within this interval. Thus, the probability remains that the cyclin D1/PRAD1 gene is the bcl-1 oncogene. Although bcl-1 often co-amplifies with other genes on 11q13 (such as EMS-1, FGF3, FGF4, int-2, and hst-1), cyclin D1 and EMS-1 are the only proteins, so far, overexpressed as a result.<sup>36 39 40</sup>

#### Cyclin D1 expression in human cancers

In addition to parathyroid adenomas, increased cyclin D1 expression has been shown in a number of primary human tumours and cell lines. In general, primary tumours provide more reliable information as it is often difficult to determine when the amplification occurred during the development of the cell line. This is because other influences, which cause the cells to proliferate at faster rates, may upregulate cyclin D1 expression. It should be remembered that although increased cyclin D1 protein expression correlates in most instances with amplification of the CCND1 gene, this is not always the case. In some tumours there is an increased cyclin D1 RNA and/or protein without apparent gene amplification, suggesting that other cellular genes (such as the retinoblastoma gene) may impact on the protein expression of cyclin D1,<sup>41</sup> although all the

mechanisms have not yet been satisfactorily elucidated. DNA amplification is the most frequent abnormality affecting the CCND1 gene. Furthermore, no major abnormality in the coding region of the cyclin D1 gene has been detected<sup>42-43</sup> suggesting that it is the normal gene product that contributes to tumorigenesis.

#### MANTLE CELL LYMPHOMAS

All or almost all mantle cell (centrocytic) lymphomas in several studies<sup>35, 44-51</sup> have raised activity of cyclin D1, even in cases in which no rearrangement at 11q13 was found.<sup>52</sup> Generally, however, positive nuclear staining with monoclonal antibody to the cyclin D1 protein correlates with amplification of the CCND1 gene as well as mRNA.<sup>53</sup> It has been suggested that expression of cyclin D1 by lymphocytes in the mantle zone impairs the capacity of these cells to exit the cell cycle and to differentiate into mature plasma cells.<sup>54</sup> This pathogenetic theory is contradicted by a recent finding of cyclin D1 protein expression in 26% of plasma cell neoplasms, however, the same study<sup>55</sup> supports a relation between mantle cells, plasma cells, and their corresponding neoplasms. An international lymphoma consensus<sup>56</sup> acknowledged the importance of chromosome 11q13 translocation and increased cyclin D1 expression in mantle cell lymphomas; in the future it may be elevated to a defining characteristic, given the high sensitivity and relative specificity of this molecule in mantle cell lymphoma compared with other B cell neoplasms.<sup>45-57</sup> Cyclin D1 protein expression and bcl-1 gene rearrangement has been identified as a key component in the diagnosis of the blastoid variant<sup>58</sup> of mantle cell lymphoma as well as in an entity closely related to mantle cell lymphoma—multiple lymphomatous polyposis.<sup>59</sup>

#### BREAST CANCERS

About half of all invasive breast cancers<sup>60-63</sup> have raised expression of cyclin D1 compared with normal epithelium, although the figure for gene amplification averages around 13%.<sup>41</sup> Some earlier studies<sup>64-67</sup> failed to find any significant association between 11q13 amplification and oestrogen receptor positive cancers, but a wealth of material<sup>68-77</sup> has now accumulated supporting a correlation between cyclin D1 gene amplification and/or protein overexpression with oestrogen receptor positive tumours. Studies in mice<sup>78-79</sup> and man<sup>80</sup> link cyclin D1 to steroid induced proliferation of mammary epithelial cells. In fact, cyclin D1 appears to be an independent activator of the oestrogen receptor.<sup>81</sup> Despite the correlation with oestrogen receptor status, there is lack of agreement as to the prognostic significance of cyclin D1 in breast cancers in general. More work needs to be done to identify the subsets of patients in whom cyclin D1 may play a more prominent role. It seems that cyclin D1 is more important in node positive,<sup>74</sup> well differentiated, and particularly lobular, varieties than other types of invasive breast cancer.<sup>75-77</sup> In a recent paper<sup>82</sup> examining the role of cyclin D1 in ductal carcinoma in situ (DCIS), high grade

lesions (graded according to the Silverstein system<sup>83</sup>) were more likely to show gene amplification but demonstrated lower percentages of nuclei expressing cyclin D1 protein than low grade lesions, which suggests that mechanisms other than gene amplification may be responsible for increased cyclin D1 protein. In this situation, assessment of cyclin D1 protein in combination with pRB may provide more useful information.<sup>84-86</sup> The publication about cyclin D1 in DCIS followed a previous paper<sup>87</sup> by the same group in which overexpression of cyclin D mRNA, determined by *in situ* hybridisation, was able to distinguish DCIS from atypical ductal hyperplasia and other lesions associated with a low risk of progression to invasive carcinoma.

#### HEAD AND NECK SQUAMOUS CELL CARCINOMAS

A range of 35% to 64% of head and neck squamous carcinomas<sup>88-93</sup> (squamous carcinomas in the oral cavity, nasopharynx, pharynx, hypopharynx, and larynx) show overexpression of cyclin D1 and/or CCND1 amplification. Overexpression of cyclin D1 in the initial surgical specimens corresponds not only with more frequent recurrence<sup>91</sup> but also with more advanced disease, lymph node involvement, and reduced overall survival.<sup>89-92-93</sup> In one study of squamous carcinomas of the larynx, a correlation among cyclin D1 gene amplification, mRNA overexpression, and tumour progression, was shown in a cohort of 46 patients.<sup>89</sup> This and another study<sup>92</sup> demonstrated a significant association between molecular abnormalities of the cyclin D1 gene and pathological measures of poor prognosis. Recently, overexpression of cyclin D1 protein in resection material from head and neck squamous carcinomas was found to be an independent prognostic factor<sup>93</sup> a finding that has been confirmed.<sup>94</sup> In Japanese hypopharyngeal squamous cell carcinomas, cyclin D1 gene amplification and protein overexpression correlated not only with prognosis but were also useful in identifying optimum treatment regimens.<sup>95</sup> Cyclin D1 negative tumours responded particularly well to multimodality treatment in these tumours.

#### OESOPHAGEAL CANCERS

In approximately 30% of oesophageal cancers, amplification<sup>96</sup> and overexpression<sup>97-99</sup> of cyclin D1 have been demonstrated, with several studies showing an association with increased mortality.<sup>99-101</sup> Also, the ability of antisense to cyclin D1 to reverse the transformed phenotype of oesophageal cancer cells<sup>102</sup> provides strong supporting evidence for the molecule's role in cancers at this site. Our group<sup>103</sup> has demonstrated recently an association between cyclin D1 protein and pRB expression, a finding that is in line with the results of another study in a similarly high incidence area.<sup>98</sup> Although our results supported an association between cyclin D1, pRB staining, and aggressive behaviour, the exact nature of this interaction will need to be clarified.

## HEPATOCELLULAR CARCINOMAS

Amplification and raised protein concentrations have been observed in 10% of hepatocellular carcinomas.<sup>104 105</sup> Among other hypotheses, it has been suggested that hepatitis B or C viral integration within the cyclin D1 gene or one of its adjacent regulator sequences may be a mechanism in malignant transformation.<sup>106</sup> In some reports<sup>107 108</sup> the hepatitis B viral genome was detected on chromosome 13 at an upstream site close to the CCND1 gene. Another study<sup>105</sup> showed that cyclin D1 amplification was restricted to hepatocellular carcinomas that contained the hepatitis B or C virus. Although the evidence is scant, the possibility of an interaction between these viruses and cyclin D1 is an intriguing one.

## COLORECTAL CANCERS

Despite an initial report<sup>109</sup> using cell lines that suggested that cyclin D1 was not an important factor in colorectal adenocarcinomas, recent work has enlightened this previously dark area. Not only does cyclin D1 overexpression occur as an early event in tumour progression, it may also be an independent prognostic factor.<sup>110</sup> There is increased nuclear immunostaining in adenomatous polyps and adenocarcinomas, but not in adjacent normal, transitional or hyperplastic mucosa. These findings apply to both sporadic<sup>111</sup> and familial forms<sup>112</sup> of colon cancer. Furthermore, as has been demonstrated in the oesophagus, antisense to cyclin D1 inhibits the growth and tumorigenicity of colon cancer cells.<sup>113</sup>

## GENITOURINARY CANCERS

Amplification of 11q13 has been demonstrated in between 6% and 21%<sup>114-116</sup> of transitional cell cancers of the urinary bladder, although nuclear accumulation of the protein appears in a much greater percentage of cases. Alterations in cyclin D1 appear to be an early event in tumorigenesis of the urinary bladder, but the prognostic significance of amplification and overexpression remain to be determined. While one study observed a significant relation between cyclin D1 overexpression and low tumour grade as well as T classification,<sup>117</sup> these findings were not duplicated in a similar study.<sup>118</sup> Abnormalities of cyclin D1 are also common in both vulval and cervical squamous cell carcinomas.<sup>119</sup> At these sites, cyclin D1 appears to inactivate pRB in a similar manner to oncogenic human papillomavirus genotypes. Thus, it seems that in vulval and cervical squamous carcinomas, human papillomavirus proteins can circumvent cellular requirements for cyclin D1<sup>120</sup> or vice versa.

In endometrial carcinomas, 11q13 amplification is exceedingly rare, but about 40% of cases show aberrant accumulation of cyclin D1.<sup>121</sup> Again, the effect of other genes is the likely explanation for this phenomenon, although in this case the interaction of cyclin D1 with p53<sup>122</sup> appears to be more important than with pRB. Because cyclin D1 can activate the oestrogen receptor independently,<sup>51</sup> if this molecule were also overexpressed in endometrial hyperplasia it would provide a useful link with

known pathogenetic mechanisms. To the best of our knowledge, this has not yet been studied.

In epithelial ovarian cancers, abnormalities of cyclin D1 are early events in the progression to malignancy, and they may be associated with the degree of transformation.<sup>123</sup> There is a strong positive correlation between cyclin D1 and c-Ki-ras immunopositivity,<sup>124</sup> but no correlation with the c-erb-B2 oncogene.<sup>125</sup> The relation with the Ras proto-oncogene is important because it has been shown that inactivation of Ras in cycling cells causes a decline in cyclin D1 protein, accumulation of the hypophosphorylated, growth suppressive form of pRB, and G<sub>1</sub> arrest.<sup>126 127</sup> Strangely, the relation between cyclin D1 amplification and oestrogen is not as clear cut as has been demonstrated in the breast.<sup>75 125</sup>

## LUNG CANCERS

Studies have shown a higher frequency of bcl-1 gene amplification in squamous cell carcinomas of the lung than in other types of non-small cell lung cancer,<sup>128</sup> and an association with poor grade and high Ki-67 labelling index.<sup>129</sup> However, current research is contradictory as to the prognostic usefulness of detecting cyclin D1 protein overexpression.<sup>130-132</sup> From studies of the resection margin epithelia of lung cancer patients, compared with non-smoking controls, it seems that genetic alteration of cyclin D1 is an early event in non-small cell lung cancer,<sup>133</sup> and therefore an attractive therapeutic target.

## SKIN CANCERS

Compared to normal skin and benign lesions, cyclin D1 protein expression is significantly greater in various malignant skin tumours, including squamous cell carcinomas, melanomas, and malignant fibrous histiocytomas.<sup>134</sup> Studies of chemically induced squamous cell carcinomas in mice also implicate cyclin D1 (and other G<sub>1</sub> cyclins) in the process of carcinogenesis.<sup>135</sup>

## SARCOMAS

Amplification of the cyclin D1 gene has been detected in a small percentage of a variety of sarcomas,<sup>136</sup> but the increased cyclin D1 protein expression in at least some cases may be due to a mutant protein with greater stability.<sup>137</sup>

## OTHER SITES AND MALIGNANCIES

Central nervous system malignancies such as astrocytomas<sup>138</sup> and glioblastomas<sup>139</sup> are not exempt from cyclin D1 amplification or protein overexpression, nor are gastric adenocarcinomas,<sup>140 141</sup> pancreatic adenocarcinomas (may be associated with a poor prognosis),<sup>142</sup> or squamous carcinomas of the gall bladder.<sup>143</sup> Only a few human tumours are still holding out against the storm of cyclin D1 research. These include pituitary tumours,<sup>144</sup> renal cell tumours,<sup>144</sup> prostate carcinoma,<sup>145</sup> and many haemopoietic malignancies.<sup>57</sup> However, with some of these tumours there are possible links with cyclin D1. For example, the 11q13 region (although apparently not the CCND1 gene) is important in pituitary neoplasms of MEN-1,<sup>146</sup>

and cyclins including cyclin D1 are important in renal development.<sup>147</sup> Such is the ubiquity of cyclin D1's involvement in neoplasia that some authors are touting this molecule as the next "molecule of the year".<sup>148 149</sup>

### Conclusion

The role of cyclin D1 in many human neoplasms supports the importance of cell cycle alterations in carcinogenesis. Cyclin D1 is not just a research tool. It is a useful aid in the diagnosis of mantle cell lymphoma. In other cancers, cyclin D1 may provide prognostic information important in the management of patients with these diseases. In the future, the cyclin D1 gene and/or protein may be a site for immunotherapy.<sup>113 150 151</sup> Before such treatments are possible, the full significance of this molecule in neoplasia needs to be examined. In the words of Winston Churchill: "It is a mistake to look too far ahead. Only one link in the chain of destiny can be handled at a time."

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