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Journals Club

Review of: Short term cyclin D1 overexpression induces centrosome amplification, mitotic spindle abnormalities, and aneuploidy

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C. J. Nelsen, R. Kuriyama, B. Hirsch, V. C. Negron, W. L. Lingle, M. M. Goggin, M. W. Stanley, J. H. Albrecht. Short term cyclin D1 overexpression induces centrosome amplification, mitotic spindle abnormalities, and aneuploidy. ***Journal of Biological Chemistry*** 2005; **280**: 768–76.

Abstract of the original article
In normal cells, cyclin D1 is induced by growth factors and promotes progression through the G1 phase of the cell cycle. Cyclin D1 is also an oncogene that is thought to act primarily by bypassing the requirement for mitogens during the G1 phase. Studies of clinical tumors have found that cyclin D1 overexpression is associated with chromosome abnormalities, although a causal effect has not been established in experimental systems. In this study, we found that transient expression of cyclin D1 in normal hepatocytes *in vivo* triggered dysplastic mitoses, accumulation of supernumerary centrosomes, abnormalities of the mitotic spindle, and marked chromosome changes within several days. This was associated with up-regulation of checkpoint genes p53 and p21 as well as hepatocyte apoptosis in the liver. Transient transfection of cyclin D1 also induced centrosome and mitotic spindle abnormalities in breast epithelial cells, suggesting that this may be a generalized effect. These results indicate that cyclin D1 can induce deregulation of the mitotic apparatus and aneuploidy, effects that could contribute to the role of this oncogene in malignancy.

Review

Regulation of cyclin D1 is central to both steroid and growth factor-induced mitogenesis in mammary epithelial cells and increased cyclin D1 expression has been implicated in mammary carcinogenesis [1]. Cyclin D1 activates two cyclin-dependent kinases (CDKs), Cdk4 and Cdk6, that regulate progress from G_1 phase into S phase. Increased cyclin D1-Cdk4/6 levels can also indirectly activate another G_1 CDK, Cdk2, via sequestration of the CDK-inhibitory proteins $p21^{WAF1/Cip1}$ and $p27^{Kip1}$. Phosphorylation of pRB, the product of the retinoblastoma susceptibility gene, by cyclin D1-Cdk4/6 and cyclin E-Cdk2 results in derepression of E2F-mediated transcription, and promotes cell cycle progression.

Cyclin D1's role in the control of cell cycle progression and the location of the cyclin D1 gene at chromosome 11q13, a locus that is frequently amplified in breast and other cancers, led to early investigations of its role as a potential mammary oncogene. These showed that cyclin D1 overexpression occurs in approximately 45% of breast cancers and that overexpression of cyclin D1 in the mammary gland of transgenic mice led to hyperplasia and eventual development of carcinoma [1]. A prevailing view has

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been that the ability of cyclin D1 to promote proliferation by activating CDKs likely accounts for its ability to promote tumour development. However, cyclin D1 levels do not correlate with Cdk4 activity in breast cancer cell lines [2], nor do they correlate with other markers of proliferation in human breast cancer tissue (reviewed in [3]), suggesting that this simple model may not be able to fully account for cyclin D1's activity as an oncogene. As well as Cdk4/6, cyclin D1 also interacts with a number of transcriptional regulators including the oestrogen receptor [4] and its ability to act as a transcriptional cofactor provides an additional potentially oncogenic function that is independent of Cdk4 binding and hence separate from its role in cell cycle progression. Expression of a point mutant of cyclin D1 that is unable to activate Cdk4 induced a set of genes that were also frequently co-expressed with cyclin D1 in primary human cancers, implicating transcriptional regulation as an alternative mechanism for cyclin D1-mediated oncogenesis [5]. Nelsen *et al.* [6] now provide evidence for a further mechanism of cyclin D1 action that may impact on its oncogenic capacity: induction of centrosome abnormalities.

Centrosomes are the microtubule-organising centres of the cell. Increases in centrosome number can cause the formation of multipolar spindles and consequent chromosome mis-segregation during mitosis, consistent with the hypothesis that the frequent abnormalities in centrosome number observed in human cancers, including breast cancer, may be linked with aneuploidy [7,8]. In their recent report Nelsen *et al.* [6] have transiently transfected hepatocytes *in vivo* using a cyclin D1-expressing adenovirus. Within 6 days of cyclin D1 adenovirus injection they observed abnormal mitoses with multipolar spindles, and cultures of explanted hepatocytes displayed increased centrosome numbers [6]. Flow cytometry of these cultures revealed a majority of cells with DNA content of $\geqslant$4 N, compared with the minor population of such cells in control cultures [6]. Analysis performed 2 days after adenoviral infection, when spindle morphology was still normal but the centrosome number had increased, showed a similar increase in the proportion of cells with DNA content of $\geqslant$4 N [6]. This was not simply due to induction of tetraploidy since cyclin D1 overexpression was associated with both numerical and structural chromosome abnormalities. Not unexpectedly, these abnormalities led to increased apoptosis, but nonetheless cells with abnormal numbers of centrosomes were apparent 4 months after adenoviral expression of cyclin D1, a time-point when cyclin D1 expression was no longer detectable [6]. Collectively these data indicate that cyclin D1 overexpression can cause centrosomal and chromosomal abnormalities that are not necessarily incompatible with ongoing proliferation. Importantly, Nelsen *et al.* go on to show that infection of cultured human breast epithelial cells with cyclin D1 adenoviruses also induces centrosome abnormalities [6]. Abnormalities in centrosomal size and/or number are correlated with chromosomal instability and are common in both *in situ* and invasive breast cancers [8], and altered cyclin D1 expression is an early event in breast cancer [1], so these new data raise the possibility that one way in which cyclin D1 overexpression may contribute to the development or progression of breast cancer is by inducing centrosomal or chromosomal abnormalities, although clearly further experimentation is necessary to substantiate this hypothesis.

One issue that impacts on the relevance of these data to breast and other cyclin D1-overexpressing cancers is whether the level of overexpression achieved in these experiments is comparable with the relatively modest cyclin D1 overexpression reached in these cancers. In an earlier publication, Nelsen *et al.* show that the maximal cyclin D1 level, achieved 2 days after adenovirus injection, is several fold greater than that after partial hepatectomy [9] although likely still within the range expected in cyclin D1-overexpressing cancers. Approximately half of the adenovirally-expressed cyclin D1 was present in large molecular mass complexes that did not appear to contain Cdk4 or Cdk6 [9]. These complexes were barely detectable after partial hepatectomy and thus their formation may largely be driven by supraphysiological levels of cyclin D1.

The observation that a molecule better known for its role in cell cycle control may also be involved in centrosomal replication is not unprecedented. To ensure that the centrosome is replicated once and only once in each cell cycle, there is close co-ordination between the control of centrosomal duplication and DNA replication. There is a clear role for aberrations in the p53 pathway in centrosomal amplification, probably through failure of the G_1/S checkpoint, and studies with viral oncoproteins have implicated the RB pathway in restricting centrosome duplication to once per cell cycle [7,8]. Centrosome duplication requires Cdk2 activation, and a proportion of cyclin E localises to the centrosome [7,10]. However, although cyclin E overexpression in fibroblasts or breast epithelial cells caused chromosome instability and polyploidy, this was not associated with abnormal centrosome numbers [11]. Similarly, Nelsen *et al.* [6] found no effect of cyclin E overexpression on centrosome numbers or spindle morphology in hepatocytes, in contrast to the effects of cyclin D1.

The intriguing observations of Nelsen *et al.* raise a number of questions about potential mechanisms

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whereby cyclin D1 might affect centrosomal replication. Is this effect mediated by a direct effect at the centrosome, or indirectly, perhaps by uncoupling the centrosomal duplication and DNA replication cycles? The authors have not examined the subcellular localisation of the overexpressed cyclin D1, so it is unclear whether a direct action is likely. Given the evidence for both CDK-dependent and -independent functions of cyclin D1, do its effects on centrosomal replication require CDK activation? In this context it will be particularly interesting to define the composition and potential functions of the high molecular weight cyclin D1 complexes apparently lacking Cdk4 and Cdk6 that form after cyclin D1 overexpression [9]. The effects of pRB deletion in hepatocytes include polyploidy but not centrosome amplification [12], indicating that loss of this essential target of cyclin D-dependent CDKs is not equivalent to cyclin D1 overexpression and thus suggesting that the effects of cyclin D1 on centrosome number may not be wholly mediated by the RB pathway. Whatever the mechanism, the observations of Nelsen *et al.* suggest new avenues for investigation that may be relevant to understanding the role of cyclin D1 in mammary carcinogenesis.

References

1. Sutherland RL, Musgrove EA. Cyclins and breast cancer. *J Mammary Gland Biol Neoplasia* 2004; **9**: 95–104.
2. Sweeney KJ, Swarbrick A, Sutherland RL, Musgrove EA. Lack of relationship between CDK activity and G_1 cyclin expression in breast cancer cells. *Oncogene* 1998; **16**: 2865–2878.
3. Ewen ME, Lamb J. The activities of cyclin D1 that drive tumorigenesis. *Trend Mol Med* 2004; **10**: 158–162.
4. Coqueret O. Linking cyclins to transcriptional control. *Gene* 2002; **299**: 35–55.
5. Lamb J, Ramaswamy S, Ford HL, *et al.* A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. *Cell* 2003; **114**: 323–334.
6. Nelsen CJ, Kuriyama R, Hirsch B, *et al.* Short term cyclin D1 overexpression induces centrosome amplification, mitotic spindle abnormalities, and aneuploidy. *J Biol Chem* 2005; **280**: 768–776.
7. Nigg EA. Centrosome aberrations: cause or consequence of cancer progression? *Nat Rev Cancer* 2002; **2**: 815–825.
8. Salisbury JL, D'Assoro AB, Lingle WL. Centrosome amplification and the origin of chromosomal instability in breast cancer. *J Mammary Gland Biol Neoplasia* 2004; **9**: 275–283.
9. Nelsen CJ, Rickheim DG, Timchenko NA, *et al.* Transient expression of cyclin D1 is sufficient to promote hepatocyte replication and liver growth *in vivo*. *Cancer Res* 2001; **61**: 8564–8568.
10. Matsumoto Y, Maller JL. A centrosomal localization signal in cyclin E required for Cdk2-independent S phase entry. *Science* 2004; **306**: 885–888.
11. Spruck CH, Won KA, Reed SI. Deregulated cyclin E induces chromosome instability. *Nature* 1999; **401**: 297–300.
12. Mayhew CN, Bosco EE, Fox SR, *et al.* Liver-specific pRB loss results in ectopic cell cycle entry and aberrant ploidy. *Cancer Res* 2005; **65**: 4568–4577.

https://doi.org/10.1017/S1470903105004463 Published online by Cambridge University Press