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$p27^{Kip1}$ and $p18^{INK4c}$ in T-47D cell cycle arrest

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Mike Davies,[Aff1]

Aff1 Clatterbridge Cancer Research Trust, UK

Keywords

CDK, cell cycle, cyclin, $p18^{INK4c}$, $p27^{Kip1}$, progestin, T-47D

Introduction

Progesterone plays an important role in the regulation of proliferation and differentiation in the normal breast by cell-cycle phase-specific actions. Progestin treatment of breast cancer cells *in vitro* results in transient stimulation followed by inhibition of proliferation. These effects are specific to the G1 phase of the cell cycle, implicating G1 cyclins, CDKs and CDK inhibitors as mediators. Cell cycle arrest is preceded by decreased cyclin abundance and CDK activity and increased association of $p27^{Kip1}$ with Cdk4-containing complexes and cyclin E-Cdk2 complexes.

Aims

To investigate the contribution of G1 cell cycle proteins to progestin-mediated inhibition of human breast cancer cell proliferation, including the relative importance of $p21^{Cip1}$, $p27^{Kip1}$ and members of the INK4 family of CDK inhibitors.

Comments

This paper clearly illustrates the complex nature of G1 cell cycle regulation and demonstrates the need to consider not just relative levels of cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors, but also their different associations and activities. The interplay between the Kip/Cip and INK4 families of CDK inhibitors is particularly interesting. Whether the control mechanisms explored here are typical of normal cells *in vivo*, or are modulated by the use of cancer cells in tissue culture, is yet to be discerned.

Methods

T-47D cells were grown in culture and treated with synthetic progestin ORG2058 or vehicle control for 24 h prior to preparation of cell lysates. Immunoprecipitation, gel filtration chromatography, western blotting and 2D gel electrophoresis were used in combination with recombinant proteins, kinase assays and RNase protection assays to assess the relative levels of cell cycle related mRNA, protein, protein-protein interaction and CDK activity.

Results

Following ORG2058 treatment, cyclin E-Cdk2 is complexed with $p27^{Kip1}$ in a ~200 kDa, kinase-inactive form. The majority of cyclin D1/-Cdk4/6 complexes are bound by $p27^{Kip1}$, and to a lesser extent $p21^{Cip1}$, without progestin treatment, with some increase in these complexes following treatment. The inability of $p27^{Kip1}$ to inhibit Cdk4 was confirmed *in vitro*by incubation with recombinant hexahistidine-tagged recombinant p27.

Analysis of $p27^{Kip1}$ isoforms in treated and untreated cells indicated that modification of $p27^{Kip1}$ does not account for the effect of ORG2058 in cyclin-CDK complexes. However, addition of ~34-182 pM recombinant His_6-p27 to cell lysates *in vitro* was capable of mimicking progestin-stimulated inhibition of cyclin E-Cdk2. Recombinant His_6-p27 was also capable of recapitulating the shift in size from ~120 kDa to ~200 kDa seen following ORG2058 treatment.

Whilst progestin treatment results in decreased levels of cyclins D1 and D3, neither this nor the increased association with $p27^{Kip1}$ is sufficient to explain the ablation of Cdk4 kinase activity. Levels of $p18^{INK4c}$ and $p15^{INK4b}$ increased in cells following ORG2058 treatment. Treatment also results in association of Cdk4 and Cdk6 with $p18^{INK4c}$. Cdk6 was found to be bound to $p18^{INK4c}$ following progestin treatment, but a significant amount of Cdk6 was bound to $p18^{INK4c}$ even in untreated cells.One mode of action of $p18^{INK4c}$ is to inhibit Cdk2 by releasing $p27^{Kip1}$ from Cdk4/6 complexes. Progestins were shown to cause a significant, but transient increase in $p18^{INK4c}$ mRNA, suggesting the increase seen in $p18^{INK4c}$ protein was due to transcriptional stimulation. The same was not true for other members of the INK4 family or $p27^{Kip1}$.

Discussion

The inhibition of T-47D cell proliferation seen on long-term treatment with progestins is the result of a series of changes in protein expression and interaction among the G1 cyclins, CDKs and CDK inhibitors. Whilst cyclin E-Cdk2 complexes are inhibited by association with $p27^{Kip1}$, the same is not true for cyclin D-Cdk4/6 complexes. Rather, in addition to decreases in cyclin D levels, Cdk4/6 activity is inhibited by association with $p18^{INK4c}$ which displaces $p27^{Kip1}$. The subsequent redistribution of $p27^{Kip1}$contributes to inhibition of Cdk2.

References

1. Swarbick A, Lee CSL, Sutherland RL, Musgrove EA: Cooperation of $p27^{Kip1}$ and $p18^{INK4c}$in progestin-mediated cell cycle arrest in T-47D breast cancer cells. Mol Cell Biol. 2000, 20: 2581-2591.