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Effects of flavopiridol on apoptosis and c-*erb*B2 in breast cancer cells

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Keywords

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Introduction

Flavopiridol is a flavone with known potent inhibitory effects on a number of cyclin-dependent kinases. When tested against a number of human cancer cell lines, it demonstrated significant growth-inhibitory activity, both *in vitro* and in the mouse xenograft model. Flavopiridol is already being tested in phase I human clinical trials; however, its exact anticancer molecular mechanisms remain to be determined. Potentially important mechanisms include the induction of apoptosis, which is partly mediated through the interactions of the competing proteins Bcl-2 and Bax. c-*erb*B-2 is a key gene involved in determining breast cancer aggressiveness including the ability to metastasise. Overexpression of the *c-erb*B-2 gene has been correlated with invasion and metastasis ability of breast tumours and one of the key mechanisms through which this is achieved is increased production and secretion of matrix metalloproteinases (MMPs).

Aims

To investigate whether flavopiridol could be effective in inhibiting the growth of breast cancer cell lines, and whether differing levels of c-*erb*B-2 expression impacted upon this effect. To determine whether flavopiridol can induce apoptosis and effect MMP secretion and invasion.

Comments

This study looked at the molecular effects of a promising new anti-cancer agent (flavopiridol) on breast cancer cell lines. It was a well-conducted study using standard and accepted methods of assessing antitumour growth effects, degree of apoptosis and cellular invasion. It appears that flavopiridol has an impact at all these levels, which makes it an attractive agent to assess clinically. Obviously it will be crucial to determine its toxicity profile, which may limit its clinical utility. Unfortunately this study had

limited scope with regards to analysis of the molecular effects of flavopiridol and it is hoped that further work investigating this area will be performed. More complete knowledge of how flavopiridol impacts upon the cell cycle, cellular arrest and apoptosis will aid in determining where flavopiridol may best fit in our breast cancer therapeutic strategies.

Methods

Four cell lines were cultured: the MDA-MB-435 human breast cancer cell line (parental cell line); two transfectant cell lines (eB1 - expressing c-*erb*B-2 258x higher, and eB4 - expressing c-*erb*B-2 165x higher than the parent cell line); and one control cell line. Growing cell lines were then exposed for 24 h to either flavopiridol (at 70, 150 and 300 nM) or DMSO (vehicle control). Cells were then harvested, stained and counted. Protein extraction and western blot analysis was also performed measuring Bcl-2, Bax, c-erbB-2 and β-actin levels. Apoptosis was measured by DNA ladder formation and by analysis of cleavage of CPP32 (caspase-3) and PARP (poly [ADP-ribose] polymerase). MMP activity was assayed using gelatin zymography and a cell invasion assay was performed using a standard matrigel invasion assay procedure.

Results

Flavopiridol was found to inhibit the growth of the parental cell line in a dose-dependent manner. Similar growth inhibition was also seen in the transfected cell lines and there was no evidence that flavopiridol activity was influenced by the level of c-*erb*B-2 expression. Exposure to 300 nM flavopiridol resulted in apoptosis in all these cell lines. Apoptosis was clearly evident by 24 h after treatment and modest upregulation of Bax and downregulation of Bcl-2 was seen. These effects were not influenced by c-*erb*B-2 overexpression. Flavopiridol induced a significant downregulation of *c-erb*B-2 with up to a 90% decrease in the level of c-erbB-2 when compared to untreated controls. Flavopiridol also inhibited the secretion of MMPs 2 and 9 and cell invasion within 18 h of exposure. The impact upon cell invasion was more pronounced in the parental cell line compared to the c-*erb*B-2 overexpressing cell lines.

Discussion

This study found that flavopiridol inhibits the growth of MDA-MB-435 breast cancer cells whether or not c-*erb*B-2 was overexpressed, possibly by inducing cellular apoptosis secondary to an increased Bax : Bcl-2 ratio. Exposure to flavopiridol also inhibited cellular invasion. The mechanism for this may be downregulation of expression of c-*erb*B-2 leading to a decrease in MMP secretion, which in turn leads to a decrease in cellular invasion. However, it is also possible that flavopiridol can inhibit MMP

secretion directly. Since flavopiridol appears to act directly against cancer cells by inducing apoptosis, in addition to preventing cellular invasion and spread, it may be an effective chemotherapeutic and/or preventive agent against breast cancer.

References

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