Effect of Bcl-xL inhibitors on LNCaP cells’ response to apoptosis induced by inhibition of PI3K

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ABSTRACT

Resistance to apoptosis induced by the inhibitor of PI3K, LY294002(LY) in the prostate carcinoma line, LNCaP when incubated with the synthetic androgen R1881 is attributed to increased Bcl-xL expression. A range of Bcl-xL inhibitors and a Bcl-2 inhibitor were tested for their ability to abolish R1881-confferred protection from LY-induced apoptosis. Of the inhibitors tested, only the green tea extract, EGCG and the Bcl-2 inhibitor, YC137 were able to abolish this androgen-mediated protection from LY. None of the BH3 structural analogs or gossypol demonstrated such capacity. Our findings suggest that the Bak-Bcl-xL binding which sequesters Bak to effect survival may be of a more specific nature than can be concluded from Bak BH3 peptide and Bax displacement assays cited in current literature, and that a precise inhibition of Bak binding to Bcl-xL is required for Bcl-xL conferred survival to be abolished. This may also be achieved by specifically inhibiting the phosphorylated form of Bcl-xL as suggested by the success of EGCG in abolishing R1881-conferred protection. The ability of YC137 to remove this androgen-mediated protection is possibly due to its interference of the STAT3 pathway shown to be required for R1881-induced upregulation of Bcl-xL expression in our signalling model for LNCaP.

INTRODUCTION

Cancer cells are characterised by a persistent survival ability, often attributed to an imbalance in activity of pro-and anti-apoptotic members Bcl-2. Proapoptotic members of the Bax family (Bax and Bak) oligomerize with the help of BH3-only family to release cytochrome c and apoptotic proteins from the mitochondria (Reed, 2006) which trigger downstream apoptotic proteins such as caspases. Executioner caspase 3 activates specific proteins for the transit to an apoptotic phase. On the other hand, survival is promoted by anti-apoptotic members such as Bcl-xL which antagonize their pro-apoptotic cousins. Reports linked Bcl-xL with the progress of prostate cancer (Castilla et al, 2006). Early stages of prostate cancer can be treated by androgen ablation (Huggin and Hodges, 1941). In this study, we use an androgen-dependent prostate cancer model, LNCaP (Horoszewicz et al, 1983) which contains a frameshift mutation in the PTEN gene resulting in a constitutively active PI3K. The PI3K inhibitor, LY294002(LY) induces apoptosis in LNCaP cells which can be prevented by overexpressing Bcl-xL (Yang et al, 2003) or by preincubating cells with the synthetic androgen R1881 prior to treatment with LY. In LNCaP, R1881 upregulates STAT3 resulting in increased Bcl-xL shown to antagonize Bak activation induced by LY (Teong, unpublished). In line with these, earlier work demonstrated the negating effect of si-Bcl-xL on R1881-confferred protection from LY-induced apoptosis.

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This project aims to identify Bcl-xL inhibitors with the capacity to abolish R1881-mediated protection from LY-induced apoptosis, hence opening new possibilities for the treatment of androgen-dependent prostate cancer. The Bcl-xL inhibitors tested in this study comprise BH3 structural analogs chelerythrine, BH3I-1, BH3I-2, HA14-1, as well as two polyphenolic compounds gossypol and EGCG. One Bcl-2 inhibitor, YC137, was also tested.

MATERIALS AND METHODS

The ability of the inhibitors to abolish R1881-mediated apoptosis was first analysed by looking at relative levels of mitochondrial succinate dehydrogenase (SDH) activity assumed to be proportional to the level of respiration and hence cell viability using MTT assay. Positive leads obtained from MTT assays were then used for further assays for caspase 3 activity at the specific concentrations using specific substrates that fluoresced when cleaved by caspase 3.

RESULTS

Figure 1: 30 µM EGCG may prevent R1881-confferred protection from LY-induced cell death
Figure 2: 10 µM YC137 may prevent R1881-conferred protection from LY-induced cell death

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Tests performed</th>
<th>Able to reduce/abolish R1881-conferred protection from LY-induced apoptosis</th>
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<tr>
<td><strong>BH3 structural analogs</strong></td>
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<tr>
<td>Chelerythrine</td>
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<td>BH3I-1</td>
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<td>Gossypol</td>
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<td><strong>Bcl-2 inhibitor</strong></td>
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<tr>
<td>YC137</td>
<td>MTT</td>
<td>Yes</td>
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Table 1: Summary of results and tests performed

DISCUSSION

Several reasons may be postulated for the inability of the BH3 structural analogs to abolish R1881-conferred protection. The first is their ability to displace Bak from Bcl-xL. In the literature, this capacity was assayed using a Bak BH3 domain peptide rather than the actual Bak protein. Tests showing the displacement of actual proteins from Bcl-xL were carried out using Bax in the studies on chelerythrine, BH3I-1 and BH3I-2, and our laboratory has found that Bcl-xL does not interact with Bax in LNCaP. HA14-1 had earlier been shown to sensitize LNCaP cells to apoptosis at a concentration range from 10 to 50µM (An et al, 2007), but such high concentrations were not possible in our investigation due to a significantly cytotoxic amount of DMSO solvent that was contributed by the additive use of LY, R1881 and inhibitor.

The inability of gossypol to inhibit R1881-conferred protection may be due to similar reasons as mentioned above. Gossypol in the literature was shown to inhibit the heterodimerization with SDH activity of LY-treated cells pre-incubated with R1881 was similar to that of cells not pre-incubated with R1881 when 10µM YC137 was used.
Bcl-xL with Bax and Bim (Zhang et al, 2007). There is hence a possibility that it does not inhibit the heterodimerization with Bak. While Huang et al (2006) showed that gossypol may down-regulate Bcl-xL, the study was performed on DU145 cells, a different prostate carcinoma cell line. It may thus be that this downregulation does not occur in LNCaP, or that the upregulation of Bcl-xL effected by R1881 is greater the down-regulation effected by gossypol. On the other hand, EGCG was able to abolish R1881-conferred protection from LY-induced apoptosis. This may be due to the different mechanism by which it acts, i.e. by reducing the hyperphosphorylated form of Bcl-xL (Kazi et al, 2002).

The Bcl-2 inhibitor, YC137 was shown to be effective at abolishing R1881-conferred protection from LY at 10µM, a phenomenon we have established as being due to Bcl-xL overexpression and not Bcl-2. A possible explanation may be inferred from Real et al’s (2004) study that pointed to a STAT3-dependent pathway in the YC137-mediated downregulation of Bcl-2. Our laboratory has earlier defined the upregulation of Bcl-xL by R1881 to be STAT3-dependent. Hence, an inhibition of this pathway would most likely prevent R1881 from increasing Bcl-xL expression, leading to an abolishment of its protective effect.

REFERENCES USED IN THIS ABSTRACT