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The effect of Src inhibition on ROS triggered signalling in human oesophageal squamous carcinoma cells

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Recommendations:

1) The abstract should be clear without reference to the rest of the dissertation. Thus abbreviations should be avoided or, when used, defined.
   Response:

Reactive oxygen species (ROS) have been specifically highlighted as instigators of aberrant pro-survival and proliferative signal transduction pathways in recent years. A possibility that ROS stimulates the Src-Protein Kinase B (PKB)-Glycogen Synthase Kinase (GSK)3-β pathway, a non-canonical pro-survival and anti-apoptotic pathway, was identified and investigated. Specifically, Human Oesophageal Squamous Cell Carcinoma (HOSCC) cells were exposed to oxidative conditions, Src inhibition and a combination of the two to determine the role that Src plays in the phosphorylation of PKB and GSK3-β in terms of ROS stimulation. Oxidative conditions led to a significant increase in pSrc and pPKB in only one of the 5 HOSCC cell lines being studied; however the abundances of pGSK3-β increased significantly in two of these cells lines and decreased significantly in two of the others. This indicates that oxidative conditions lead to different downstream effects in the various HOSCC cell lines, which are most likely achieved via the activation of various pathways as a result of crosstalk. Src inhibition led to a decrease in pPKB levels across all HOSCC cell lines displaying detectable levels of pPKB, however the abundance of pGSK3-β increased in these cell lines, indicating again that other pathways are at play with respect to the activation of GSK3-β in HOSCC cells. This is due to the fact that GSK3-β is a downstream effector of PKB, and should thus decrease in abundance as pPKB does. Oxidative conditions coupled with Src inhibition resulted in an increase in the abundance of pPKB and pGSK3-β in four of the five HOSCC cell lines. These results indicate that Src inhibition under oxidative conditions may lead to cell survival and proliferation via the activation of pPKB, a pivotal pro-survival protein, and subsequent inactivation of pGSK3-β, a known pro-apoptotic protein. Thus, although more than one pathway is likely to be involved in the phosphorylation of PKB and GSK3-β in HOSCC in terms of ROS, it appears as though inactive Src in HOSCC cells undergoing oxidative stress could be used as a testable marker for HOSCC – a devastating disease affecting numerous South Africans.

2) The abbreviation of Src should be listed in the list of abbreviations and symbols.
   Response: Src is not an abbreviation.

3) Unnecessary open spaces in the text should be removed throughout the dissertation.
   Response: Corrected

4) The resolution and size of figures 1.1 and 1.2 could be improved.
   Response: Corrected

5) The clear formulation of a research question or hypothesis was lacking.
   Response:

The connection between elevated levels of pSrc and squamous cell carcinomas, as well as its involvement in the activation of PKB and GSK3-β, has made it an interesting target for research in terms of HOSCC. Not only Src itself, but also pPKB and pGSK3-β, have been implicated in the formation of many tumours, including squamous cell carcinomas. These particular proteins of interest have all been recorded as being affected by reactive oxygen species (ROS). Thus, the aim of this investigation was to determine the role of Src in the phosphorylation of PKB and GSK3-β under oxidative conditions in HOSCC, in which the role of ROS and the Src-PKB pathway is relatively unknown.

The objectives were as follows:

…

6) Results 3.1. When referring to Materials and Methods section in the 1st paragraph, name the particular section number referred to. Also refer to Fig 3.1 in the 1st paragraph.
   Response: Corrected.

7) Figure 3.2 legend title: Different abundances of pSrc… Indicate the number of replicates used in each experiment in figure legends as (n=….).
   Response: Corrected.

8) Results section 3.2:
   - 1st paragraph: when referring to submillimolar or high levels of H_2O_2, be more specific; below or above what level?
     Response: *This particular concentration of H_2O_2 was used because the addition of micromillimolar levels of H_2O_2 has been found to be more effective at causing DNA lesions than millimolar concentrations. This could have a profound impact on the results.*
   - 2nd paragraph: Omit therefore and add new sentence… ~~therefore~~ allowing the cells to remain in an oxidative…
     Response: corrected.
   - 3rd paragraph: refer to figure 3.3.
     Response: corrected.

9) Page 28:
    - 1st paragraph (See Figure 3.3A)
      Response: Corrected.
    - 2nd paragraph: check font type and sentence structure. Suggestion:
      Interestingly, the abundance of pGSK3-β decreased significantly in WHCO6 and WHCO3 cells in response to ROS stimulation. WHCO1 and SNO cells responded to ROS stimulation with a significant increase in pGSK3-β abundance.
      Response: Corrected.
    - Bar chart's X-axis bar labels should be below the graph and not below zero, often over-writing bars and error bars.
      Response: Corrected.

10) Page 31, last paragraph: There was, however, a major AND significant increase.
    Response: Corrected.

11) Page 32: increases were significant in WHCO5, **WHCO6**, A431 and H1299 cells.
    Response: Corrected.

12) Page 40 2nd paragraph: Use semicolons: …cell lines (Nerwich, 2013; Shaw, 2011; Fanucchi, 2011).
    Response: Corrected.

13) The candidate should do a final literature search to ensure inclusion of the latest findings in her study field.
    Response: completed.

14) Page 8, DMEM, correct "Eagle" to read "Eagle's".
    Response: Corrected.

15) Page 21, 1st paragraph, 1st line, change "Eagle" to read "Eagle's".
    Response: Corrected.

16) Page 21, 1st paragraph, 1st line, change "Highveld Biologicals" to read "Highveld Biological".
    Response: Corrected.

17) References: Please check all journal names as they are not appropriately capitalized.
    Response: Corrected.