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Synaptonemal complex protein SYCP3 impairs mitotic recombination by interfering with BRCA2

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1st Editorial Decision 18 March 2011

Thank you for the submission of your research manuscript to EMBO reports. It was sent to three referees and we have now received reports from two of them (which you will find below). As they are in fair agreement, I have made a decision now in order to spare you from unnecessary loss of time. As you will see, although both referees find the topic of interest and in principle suitable for us, they also consider the study preliminary at this stage for publication in EMBO reports. Given that both referees provide constructive suggestions on how to make the work more conclusive, I would like to give you the opportunity to revise your manuscript.

Of important concern is the need to establish the relevance of your findings by showing expression of SYCP3 in a larger panel of tumor cell lines as well as human primary tumors. Homologous recombination should be assayed directly and in cells in S/G2 phase of the cell cycle, and referee 1's point 5 addressed (regarding the use of cisplatin and additional cell lines). There are several additional requests for clarification which would be useful to answer. However, please note that, as a short report journal, we would not require you to elucidate the mechanism of action of SYCP3 and therefore request number 6 of referee 1 would be out of the scope of the present study.

If the referee concerns mentioned above can be adequately addressed, we would be happy to accept your manuscript for publication. However, please note that it is EMBO reports policy to undergo one round of revision only and, thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, do not hesitate to get in touch with me if I can be of any assistance.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

The study by N. Hosoya et al. presents evidence for the synaptonemal complex protein SYCP3 participation in recombination processes of mitotically dividing cells, which is a novel function proposed for this protein. The authors propose that SYCP3 can exert its negative role in recombination by binding to BRCA2 and interfering with BRCA2-Rad51 interplay and Rad51 foci formation, thereby also leading to enhanced sensitivity to PARP inhibitors and increased genomic instability. The work is certainly novel and of rather broad interest, and therefore worthy of consideration for publication. On the other hand, some aspects of this work are insufficiently developed and/or unclear, and require more evidence to support the major conclusions.

Specific comments.

1. The evidence for a role in recombination is suggestive, but there is a more direct cellular assay to measure HR efficiency, based on a GFP readout - developed by Maria Jasin and commonly used in the field. It would be important to assess the impact of different levels of SYCP3 on HR in this assay.

2. The cellular models used in this work are somewhat confusing. For example, the authors often switch from one cell line to another for different assays and figures, and there is no consistency for a particular model and explanation why it is suitable. In addition, the cell line HCT116 used here is grossly aberrant in the MRN complex that is critical for upstream events of t homologous recombination (since Mre11 is mutant and the MRN complex non-functional) - this is not considered. Also, several figures are based on RPE cells, but these are never even mentioned in the material and methods...So, the models need careful attention, explanation, and also addition of some non-cancerous normal human cells, unless RPE is such a cell type.

3.It is unclear whether the interaction of SYCP3 with BRCA2 is modfied in response to DNA damage - this needs some data.

4. As some of the presented effects are relatively modest, it would be useful to focus on S/G2 cells, where recombination takes lace, and avoiding analysis of G1 cells which is often the bulk of the cell population. By using either cell synchronization or double staining using e.g. cyclin A to identify S/G2 cells, the observed effects may well become more robust and convincing

5. The PARP inhibitor sensitivity aspect is very relevant, however somewhat preliminary. It would be useful to complement the data by using also cisplatin, and extend the experiment to some additional cellular model (e.g. ovarian or breast cancer cell lines). Finally, it would be predictable that cells deficient in BRCA2 should not become more sensitive to PARP inhibitors upon SYCP expression, - is this the case?

6.From the mechanistic point of view, it is unclear whether the interaction of BRCA2 with SYCP3 is direct or indirect, and if the former, whether SYCP3 comptese with Rad51 for the same domain of BRCA2 - this can be tested using the established fragments of BRCA2.

6.

Referee #3:

Summary

The authors describe that expression of a SYCP3 transgene in immortalized human retinal pigment epithelial (RPE) cells inhibits IR induced RAD51 foci formation and impairs chromosomal integrity. SYCP3 is a component of the synaptonemal complex with no known role outside meiosis. Therefore these findings are novel. Furthermore, SYCP3 forms a complex with BRCA2, and there is reduced binding of BRCA2 with RAD51 in SYCP3 expressing RPE clones compare to mock transgenic cells. This may explain reduced RAD51 foci formation and increased sensitivity to PARP inhibitor NU1025, IR and cisplatin in these cells. Knockdown of SYCP3 in HepG2 cells increased IR induced RAD51 foci formation and decreased sensitivity to NU1025, thus confirming the results with the RPE clones. The authors suggest that treatment with PARP inhibitors may be effective in tumors that (are forced to) express SYCP3.

General impression

The article has a clear message that is well supported by most of the experimental data. SYCP3 may play a role outside myosis and reduce HR by inactivating BRCA2. This is a novel concept and could be of clinical interest in tumors expressing SYCP3. However, the authors should address a few issues relating to the possible clinical relevance of their data.

Major comments

1. An important weakness of the paper is that no firm evidence is presented for reversion of SYCP3 silencing in a notable subset of tumors or tumor cell lines. Supplementary figure 1 only shows some weak protein and/or cDNA bands in a number of tumor cell lines. The RT-PCR analysis in supplementary figure B is not quantitative and some tumor cell lines with strong bands are not present in the western blot of supplementary figure A (Jurkat, KCL22). The authors should present more convincing evidence of SYCP3 expression in primary human tumors. In addition, they should present some evidence that SYCP3 expression in tumor cells is functionally relevant. Can SYCP3 expression be used as a marker of HR deficiency? Is SYCP3 expression specifically found in certain tumor types, thereby creating a therapeutic window for HR targeted therapy? A comprehensive analysis of SYCP3 mRNA expression in available gene expression microarray data from human tumors would be informative. Furthermore, two of the three articles referenced in the Introduction section do not clearly document SYCP3 expression in tumors and Niemeyer et al. only describe RT-PCR products in ALL. More evidence for SYCP3 expression in cancer has been published by Kang et al. (Cancer Research 2010;70:3062-70) and should be referred to. Finally, the authors should at this stage not overstate the possible importance of SYCP3 status for responsiveness to HR targeting therapy. More data from patient cohorts and/or tumor models are required and speculations about the possibility to reactivate SYCP3 as a therapeutic strategy should be tempered. In sum, the authors should substantiate the 'accumulating evidence that SYCP3 is expressed in tumors' using more convincing data. The authors could also improve supplementary figure 1 by adding more normal controls, real tumor samples and including Jurkat and KCL22 cells in the protein analysis. In the absence of a comprehensive analysis of SYCP3 expression in primary human tumors, the preliminary nature of the available evidence for a role of SYCP3 in tumors should be taken into account in the discussion.

2. Figure 2: SYCP3 impairs the RAD51-dependent homologous recombination (HR) pathway in mitotic cells. This figure shows decreased IR induced RAD51 foci formation in the presence of SYCP3. Although this suggests that HR is impaired, that is not actually shown. The authors should rephrase the figure title and manuscript text accordingly. In addition, the number of cells analyzed should be mentioned as was done for the foci analysis in the supplementary data.

3. Although the RAD51 foci in figure 2 seem to be clear, NBS1 IF in figure S3 does not indicate clear foci and the BRCA1 IF pictures suggest abundant foci formation before IR which I don't understand. How can this be explained and related to the percentages of cells with more than 5 damaged induced foci? Are the pictures representative? Details on microscopy might be informative

and should be added.

Minor comment

Page 2, line 2 and page 3 line 5: 'expressed in tumor' should be 'expressed in tumors'.

1st Revision - authors' response 14 June 2011

Responses to the referee's comments

Responses to the referee #1

[Comment]
1. The evidence for a role in recombination is suggestive, but there is a more direct cellular assay to measure HR efficiency, based on a GFP readout - developed by Maria Jasin and commonly used in the field. It would be important to assess the impact of different levels of SYCP3 on HR in this assay.

[Answer]
1. We entirely agree with this comment. In order to provide direct evidence that SYCP3 impairs the homologous recombination (HR) repair pathway, we performed the DR-GFP assay developed by Maria Jasin, by transiently expressing FLAG-SYCP3 together with ISceI restriction enzyme in HeLa-DRGFP cells previously described. We found that SYCP3 expression reduces HR efficiency in a dose-dependent manner, although the presented reduction levels of the HR efficiencies upon SYCP3 expression are relatively modest, which may be due to limitations in the co-transfection efficiency in a transient transfection system. In the revised manuscript, we added the data of the DR-GFP assay in Figures 2E,F, and also added a new result section entitled "SYCP3 dose-dependently reduces HR efficiency" (page 8, lines 16~) in the text.

[Comment]
2. The cellular models used in this work are somewhat confusing. For example, the authors often switch from one cell line to another for different assays and figures, and there is no consistency for a particular model and explanation why it is suitable. In addition, the cell line HCT116 used here is grossly aberrant in the MRN complex that is critical for upstream events of homologous recombination (since Mre11 is mutant and the MRN complex non-functional) - this is not considered. Also, several figures are based on RPE cells, but these are never even mentioned in the material and methods...So, the models need careful attention, explanation, and also addition of some non-cancerous normal human cells, unless RPE is such a cell type.

[Answer]
2. We are sorry that there were not sufficient explanations about the cell lines used in the different assays in the original manuscript. RPE is a non-cancerous normal human epithelial cell line, and we used this cell line to established stable clones expressing SYCP3 or FLAG-SYCP3 to assess the effect of SYCP3 expression in normal mitotic cells. In this study, we also performed knockdown experiments using 4 different cancer lines endogenously expressing SYCP3, such as HepG2, HT1080, MCF7 and Capan-1. In the revised manuscript, we added the following phrases in the text, where the explanations of the cell lines used in the assays were not sufficient in the previous manuscript.

Page 4, lines 1~4; " We first examined the expression profiles of SYCP3 using two noncancerous normal human cells and 16 human cancer cell lines by western blot analysis. While no expression of SYCP3 was detected in the normal retinal pigmented

epithelial cell line RPE and the normal human mammary epithelial cell line HME,"

Page 5, lines 4~6; " we expressed the cDNA encoding SYCP3 in the normal human cell line RPE and established two independent clones stably expressing SYCP3"

Page 6, lines 13~15; "We therefore assessed the IR-induced foci formation of several recombination molecules including RAD51 in RPE clones stably expressing FLAGSYCP3….."

Page 9, lines 13~15; "we assessed the localization of the SYCP3 protein and key molecules involved in HR in RPE cells stably expressing FLAG-SYCP3"

Page 10, lines 15~16; "Indeed, RPE cells expressing FLAG-SYCP3 showed extreme hypersensitivity to the PARP inhibitor NU1025 (Fig 4A),"

Page 10, lines 17~20; "Reversely, reduced SYCP3 expression levels in HepG2, the human fibrosarcoma cell line HT1080 and the breast cancer cell line MCF7, increased the colony survival after treatment with 150 μM NU1025"

Page 11, lines 6~7; "silencing of SYCP3 in Capan-1 cells did not affect the sensitivity to the PARP inhibitor (supplementary Fig S6D and Fig S7D online).

Also, please note that we did not use the MRN-deficient cell line HCT116 for the assays to measure the HR repair efficiency in this study, because the results for this cell line might be greatly modified by its deficiency in the MRN complex.

[Comment]
3. It is unclear whether the interaction of SYCP3 with BRCA2 is modified in response to DNA damage - this needs some data.

[Answer]
3. We performed the pull down assay using RPE cells expressing FLAG-SYCP3 treated or untreated with 8 Gy X-ray, and found that the interaction of SYCP3 with BRCA2 is not modified in response to DNA damage. In the revised manuscript, we replaced the Figure 3B into a new one, and also added the following sentence in the text; "The interaction between SYCP3 and BRCA2 was not modified in response to IR-induced DNA damage (Fig 3B)" (Page 9, lines 18~19).

[Comment]
4. As some of the presented effects are relatively modest, it would be useful to focus on S/G2 cells, where recombination takes place, and avoiding analysis of G1 cells which is often the bulk of the cell population. By using either cell synchronization or double staining using e.g. cyclin A to identify S/G2 cells, the observed effects may well become more robust and convincing.

[Answer]
4. We agree with this comment. According to the suggestions, we examined the frequencies of IR-induced RAD51 foci-positive cells in S and G2 phases in RPE cell clones expressing FLAG-SYCP3 or mock cells as well as in HepG2 cells transfected with siRNA by double staining of RAD51 with cyclin A. Focusing on S/G2 cells, we obtained more robust effects of expression or knockdown of SYCP3 on the IR-induced foci formation. In the revised manuscript, we added these results of IR-induced RAD51 foci formation in cells stained with cyclin A in supplementary Figures S4C,D and S5C, and also described about the results as follows in the text.

Page 7, lines 4~8; "The frequency of IR-induced RAD51 foci-positive cells in cells in S and G2 phases was also examined by double staining of RAD51 with cyclin A (supplementary Fig 4C online), and a robust decrease from 96.7 ± 0.6 % in mock cells to 59.0 ± 3.6 % and 67.3 ± 6.8 % in the two FLAG-SYCP3-expressing RPE clones was observed (supplementary Fig 4D online)."

Page 8, lines 8~12; “Focusing on cells in S and G2 phases, a robust increase in the frequency of IR-induced RAD51 foci-positive cells from 53.7 ± 2.3 % to 94.3 ± 2.5% was observed after silencing SYCP3, which was rescued to 53.3 ± 3.1% in rescue experiments with the siRNA-resistant FLAG-SYCP3 construct (supplementary Fig S5C online), confirming the phenotype specificity for the SYCP3 gene.”

[Comment]
5. The PARP inhibitor sensitivity aspect is very relevant, however somewhat preliminary. It would be useful to complement the data by using also cisplatin, and extend the experiment to some additional cellular model (e.g. ovarian or breast cancer cell lines). Finally, it would be predictable that cells deficient in BRCA2 should not become more sensitive to PARP inhibitors upon SYCP expression, - is this the case?

[Answer]
5. Firstly, we examined the effect of addition of 4 μM cisplatin on hypersensitivity to the PARP inhibitor observed in FLAG-SYCP3-expressing RPE cells. We found that pretreatment with 4 μM cisplatin for 1 hour remarkably enhanced the hypersensitivity of RPE-FLAG-SYCP3-expressing cells to NU1025. In the revised manuscript, we added this result in supplementary Figure S7A, and also added the following sentence in page 10, lines 15~16; “addition of 4 μM cisplatin remarkably enhanced this hypersensitivity (supplementary Fig S7A online).”

Secondly, we also extended the knockdown experiments to some additional cancer cell lines endogenously expressing SYCP3, including the fibrosarcoma cell line HT1080 and breast cancer cell line MCF7 in addition to HepG2 (supplementary Figure S6A-C), and found that the colony survival after treatment with 150 μM NU1025 also increased after silencing SYCP3 in the additional cell lines (supplementary Figure S7B,C). In the revised manuscript, we added these results in supplementary Figures S7B and S7C, and also added the following sentence in page 10, lines 17~22; “Reversely, reduced SYCP3 expression levels in HepG2, the human fibrosarcoma cell line HT1080 and the breast cancer cell line MCF7, increased the colony survival after treatment with 150 μM NU1025 from 0.9 ± 0.77% (mean ± SD) to 7.2 ± 2.2% in HepG2 cells, from 4.0 ± 2.5 % (mean ± SD) to 17.8 ± 2.7 % in HT1080 cells, and from 7.0 ± 1.5 % (mean ± SD) to 14.0 ± 0.4 % in MCF7 cells, respectively (Fig 4B, supplementary Fig S6A-C and Fig S7B,C online).”

Finally, as predicted, it is likely to be the case that the cells naturally deficient in BRCA2 do not become more sensitive to PARP inhibitors upon SYCP expression. Silencing SYCP3 in the BRCA2-deficient Capan-1 cells, which also endogenously express SYCP3, did not alter the sensitivity of the cells to the PARP inhibitor. In the revised manuscript, we added this result in supplementary Figure S7D, and also added the following sentences in page 11, lines 1~7; “It would be predictable that the sensitivity to PARP inhibitors in cells that are naturally deficient in BRCA2 would not be affected by SYCP expression. The pancreatic cancer cell line Capan-1, which expresses SYCP3 (supplementary Fig S1A online), carries a 6174delT mutation in one BRCA2 allele accompanied by loss of the wild-type allele and is reported to be sensitive to PARP inhibitors (McCabe et al, 2005). Supporting the prediction, silencing of SYCP3 in Capan-1 cells did not affect the sensitivity to the PARP inhibitor (supplementary Fig S6D and Fig S7D online).”

[Comment]
6. From the mechanistic point of view, it is unclear whether the interaction of BRCA2 with SYCP3 is direct or indirect, and if the former, whether SYCP3 compete with Rad51 for the same domain of BRCA2 - this can be tested using the established fragments of BRCA2.

[Answer]
6. Our preliminary data demonstrated a direct association between SYCP3 and BRCA2, both by using full-length BRCA2 and fragments of BRCA2. Regarding the binding domain, we found it in a region that is not known to be involved in interaction between

BRCA2 and RAD51. While it might be possible that interaction of SYCP3 with this region of BRCA2 would regulate the interaction between BRCA2 and RAD51 by some remote effects, we should generate a mutant of BRCA2 that cannot bind SYCP3 to address this, which is under investigation and would require a lot of time considering that BRCA2 is a very large protein. Because our results with regard to the mechanistic point remain preliminary at this stage and we were told by the editor that this point would be out of the scope as a short report journal, we did not include this point in the present study. In the revised manuscript, we added the following sentence in the Discussion section; “Detailed characterization of the interaction between SYCP3 and BRCA2 would be needed to address this question entirely” (page 11, line 22~). We will hopefully address this point in the next publications.

Responses to the referee #3

[Comment]
1. An important weakness of the paper is that no firm evidence is presented for reversion of SYCP3 silencing in a notable subset of tumors or tumor cell lines. Supplementary figure 1 only shows some weak protein and/or cDNA bands in a number of tumor cell lines. The RT-PCR analysis in supplementary figure B is not quantitative and some tumor cell lines with strong bands are not present in the western blot of supplementary figure A (Jurkat, KCL22). The authors should present more convincing evidence of SYCP3 expression in primary human tumors. In addition, they should present some evidence that SYCP3 expression in tumor cells is functionally relevant. Can SYCP3 expression be used as a marker of HR deficiency? Is SYCP3 expression specifically found in certain tumor types, thereby creating a therapeutic window for HR targeted therapy? A comprehensive analysis of SYCP3 mRNA expression in available gene expression microarray data from human tumors would be informative. Furthermore, two of the three articles referenced in the Introduction section do not clearly document SYCP3 expression in tumors and Niemeyer et al. only describe RT-PCR products in ALL. More evidence for SYCP3 expression in cancer has been published by Kang et al. (Cancer Research 2010;70:3062-70) and should be referred to. Finally, the authors should at this stage not overstate the possible importance of SYCP3 status for responsiveness to HR targeting therapy. More data from patient cohorts and/or tumor models are required and speculations about the possibility to reactivate SYCP3 as a therapeutic strategy should be tempered. In sum, the authors should substantiate the 'accumulating evidence that SYCP3 is expressed in tumors' using more convincing data. The authors could also improve supplementary figure 1 by adding more normal controls, real tumor samples and including Jurkat and KCL22 cells in the protein analysis. In the absence of a comprehensive analysis of SYCP3 expression in primary human tumors, the preliminary nature of the available evidence for a role of SYCP3 in tumors should be taken into account in the discussion.

[Answer]
1. According to the referee’s suggestions, first of all, we replaced the supplementary Figure S1A with a new one, in which we showed expression analysis of the SYCP3 protein in a larger panel of tumor cell lines including Jurkat and KCL22 as well as two normal non8 cancerous cell line RPE and HME, together with normal testis as a positive control. Except for HepG2 and DU145 that showed moderate protein expression levels, the expression levels of the SYCP3 protein in cancer cell lines are low compared to that in normal testis, but in the revised manuscript we could successfully indicate the reversion of the phenotypes of impaired homologous recombination (HR) after silencing SYCP3 in a total of 4 different tumor cell lines, including HepG2, HT1080, MCF7 and Capan-1, suggesting that SYCP3 expression in tumor cells is functionally relevant and that SYCP3 expression would be used as a marker of HR deficiency.

Secondly, in order to present convincing evidence of SYCP3 expression in various primary human tumors, we analyzed expression of SYCP3 using a mRNA array that contained two duplicated spots of mRNA from 47 different tumors and 47 normal tissues from unmatched donors (supplementary Fig S2A and Table S1 online),. Significantly increased levels of SYCP3/!-actin signal ratios were observed in 1 adrenal tumor, 3 liver

tumors, 1 stomach tumor and 1 kidney tumor (supplementary Fig S2B online), indicating that SYCP3 expression is not specific to certain tumor types but is observed in tumors of various tissue origins. In the revised manuscript, we added these results for expression analysis of SYCP3 in primary tumors in page 4, lines 14~23, and page 5, lines 1~2. We also added new figures in supplementary Fig S2A, B and Table S1 online, regarding the expression analysis of SYCP3 in primary tumors. Furthermore, according to the suggestions, we deleted two of the three articles that we referenced in the Introduction section that did not clearly document SYCP3 expression in tumors, and referred to the publications by Niemeyer et al. and Kang et al. as follows; "Although SYCP3 was first considered to be a meiosis-specific protein, it has been reported to be aberrantly expressed in human leukemia and primary cervical cancers (Niemeyer et al, 2003; Kang et al, 2010)" in page 3, lines 4~7.

Finally, we agree with the comment that we should at this stage not overstate the possible importance of SYCP3 status for responsiveness to HR targeting therapy. According to the suggestion, we deleted the description about the speculations about the possibility to reactivate SYCP3 as a therapeutic strategy; "PARP inhibitors may be effective in tumors expressing SYCP3, and even in tumors not expressing SYCP3 if they can be used in combination with agents that can induce SYCP3 expression specifically in cancer cells." We also revised the last sentence in Discussion as follows; "Establishment of this new therapeutic strategy would require further analyses in large patient cohorts and tumor models." (page 12, lines 10~11)

[Comment]
2. Figure 2: SYCP3 impairs the RAD51-dependent homologous recombination (HR) pathway in mitotic cells. This figure shows decreased IR induced RAD51 foci formation in the presence of SYCP3. Although this suggests that HR is impaired, that is not actually shown. The authors should rephrase the figure title and manuscript text accordingly. In addition, the number of cells analyzed should be mentioned as was done for the foci analysis in the supplementary data.

[Answer]
2. According to the other referee's suggestions, we also directly measured the HR efficiencies in SYCP3-expressing cells with the DR-GFP assay, and found that SYCP3 reduces the HR efficiency in a dose-dependent manner. In the revised manuscript, we included this result in the new section "SYCP3 dose-dependently reduces HR efficiency" (page 8, lines 16~23, page 9, lines 1~3) and added two related figures from the DR-GFP assay in the new Figure 2 (Fig 2E and 2F), together with the decreased IR-induced RAD51 foci formation in the presence of SYCP3 in RPE cells (Figure 2A,B). Thus, we don't need to rephrase the figure title "SYCP3 impairs the RAD51-dependent homologous recombination (HR) pathway in mitotic cells" any more. In addition, in the analysis of foci formation, we analyzed a total of 100 cells per one experiment and performed three independent experients. We included the sentences "For the analyses of foci formation of RAD51, NBS1 and BRCA1, a total of 100 cells per each cell type were counted in randomly selected fields using an Olympus BX51 fluorescence microscope" in page 5, lines 11~13 of the revised supplementary information.

[Comment]
3. Although the RAD51 foci in figure 2 seem to be clear, NBS1 IF in figure S3 does not indicate clear foci and the BRCA1 IF pictures suggest abundant foci formation before IR which I don't understand. How can this be explained and related to the percentages of cells with more than 5 damaged induced foci? Are the pictures representative? Details on microscopy might be informative and should be added.

[Answer]
3. We agree with this comment. We replaced the corresponding supplementary figures S4E and S5D with new ones so that the foci of NBS1 are more apparent. We also replaced the corresponding supplementary figures S4G and S5F with new ones, because the pictures for the BRCA1 before IR in the previous version of our manuscript were inappropriate and not representative. We believe that the qualities of the figures of NBS1 and BRCA1

foci are much improved in the revised manuscript. We also added the details on microscopy in the supplementary methods as follows; “For the analyses of foci formation of RAD51, NBS1 and BRCA1, a total of 100 cells per each cell type were counted in randomly selected fields using an Olympus BX51 fluorescence microscope at 1,000 magnification. Images were captured with an Olympus DP 70 digital camera using Olympus DP controller and DP manager softwares.” (page 5, lines 11~15 in the revised supplementary information). In each (supplementary) legend for figures, the information about the scale bars is also indicated.

[Minor comment]
Page 2, line 2 and page 3 line 5: 'expressed in tumor' should be 'expressed in tumors'.

[Answer]
We revised the description in page 2, line 2 according to the referee’s comment. The description of page 3, line 5 was replaced to another sentence in the revised manuscript.

2nd Editorial Decision 18 July 2011

Thank you for the submission of your revised manuscript to our offices and please accept my apologies for the time it has taken me to return to you with a decision on your manuscript. I sent your revised study to referee #1, who has only been able to return the enclosed report today due to unforeseen circumstances. As you will see, although this referee appreciates the work performed during revision, s/he still points out a concern regarding the modest effect of SYCP3 expression on HR efficiency in the DR-GFP assay. As this was one of the main issues from the beginning, I think it should be addressed and, thus, would like to provide you an exceptional second round of revision to perform this assay in a stable cell line with the integrated reporter system, as the referee suggests. In addition, statistical analyses to assess if the differences observed are significant should be provided. Ideally, this should also be performed in a cell line expressing endogenous SYCP3, so that the effect of its knock-down can be assessed, although I feel that given all the other evidence in the manuscript, this last point would be less crucial. As we do not allow the time between initial and final decision to exceed six months, this would give you until September 13th to submit the final version of your study. I trust this will be sufficient time, please let me know otherwise.

In going through the manuscript, I have also noticed that the Materials and Methods section is now extremely succinct. Please note that the essential information of the methods required for understanding the experiments performed must be included in the main text. Please also ensure that all figure panels contain a description of the error bars and number of experiments performed, if applicable. Some of the figure panels are currently missing this information. Please note that the minimum number of independent experiments from which errors can be calculated is three. Thus, it is inappropriate to include error bars in supplementary figure 2B; both data points should be shown instead and the legend modified accordingly.

Lastly, I feel that the information included in supplementary figures 4C, 4D, 5C and 7A-D is important and should be included in the main text. To this end, I could increase the number of figures in the main text to 5. Regarding SF7, it seems appropriate to substitute current figure 4A for SF7A, add SF7B, C to Fig 4B (the three graphs can all be included in panel B) and add SF7D to Fig. 4. Please also include a mention of your initial data on the interaction between BRCA2 and SYCP3 (that it seems to be direct), as elaborated in response to referee 1's point 6. Although it would be out of the scope to pursue this aspect experimentally, it would add to the present study to give an indication of how it might be occurring.

If these last concerns can be adequately dealt with, we would be happy to accept your manuscript for publication. I look forward to seeing a final version of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

In response to my comments, the authors have performed a number of additional experiments and overall the data now better supports their original conclusion. I would still ask about the important experiment with the fluorescence/GFP -based HR assay, now presented in Fig. 2E,F. The differences observed due to the presence of SYCP3 are rather modest. Are these differences really statistically significant? It would be much better to use a stable cell line with the integrated reporter system (rather than transient co-expression approach), and many such cell lines have been published. Also, it would be useful to assess this parameter upon knock-down of SYCP3, rather than just in overexpression experiments.

2nd Revision - authors' response 11 September 2011

Responses to the referee's comments

Responses to the referee #1

[Remarks]
In response to my comments, the authors have performed a number of additional experiments and overall the data now better supports their original conclusion. I would still ask about the important experiment with the fluorescence/GFP -based HR assay, now presented in Fig. 2E,F. The differences observed due to the presence of SYCP3 are rather modest. Are these differences really statistically significant? It would be much better to use a stable cell line with the integrated reporter system (rather than transient co-expression approach), and many such cell lines have been published. Also, it would be useful to assess this parameter upon knock-down of SYCP3, rather than just in overexpression experiments.

[Answer]
We entirely agree with this comment. During revision, we performed the DR-GFP assay with several stable cell lines with the integrated reporter system, as you suggested. First, we established HeLa-DRGFP cells stably expressing exogenous FLAG-SYCP3, and showed that stable expression of FLAG-SYCP3 robustly reduces the HR efficiency, which is statistically significant. Secondly, we established RPE-FLAG-SYCP3 cells in which the DR-GFP reporter system is stably integrated, and showed that knockdown of exogenous FLAG-SYCP3 significantly recovers the HR efficiency in these cells. Lastly, we also established HepG2-DRGFP cells and MCF7-DRGFP cells, which express the endogenous SYCP3 protein, and showed that knockdown of endogenous SYCP3 in these cells significantly recovers the HR efficiency. We included all these data in the current figure 3 and described the results from page 8, line 13 to page 9, line 8. We believe that the concerns have been adequately dealt with.

3rd Editorial Decision 18 October 2011

Thank you for your patience while we contacted referee 1 once more. S/he is now happy with the study and I am very pleased to accept it for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,
Editor
EMBO Reports

