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Clarifying the role of EMSY in DNA repair in ovarian cancer

Running Title: EMSY in DNA repair in ovarian cancer

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Precis: While evidence supports EMSY's involvement in DNA repair, EMSY amplification fails to suppress RAD51 foci formation, a marker of homologous recombination DNA repair. The majority of analysis to date has been performed in cell lines, and models more closely representing patients should be studied to determine EMSY's relevance for use as a predictive biomarker for platinum and PARPi therapy responses.

Keywords: Ovarian Cancer, EMSY Amplification, DNA Repair, Homologous Recombination, EMSY Overexpression, PARP Inhibitors

One of the key characteristics of high grade epithelial ovarian cancer (OC) is that up to 50% of such cases can have defects in the homologous recombination (HR) DNA repair pathway.[1] This in turn should make the OC susceptible to DNA damaging chemotherapy, such as platinum agents, and to a potent targeted therapy called PARP inhibitors (PARPi).[2] For some of the HR defects, such as *BRCA1/2* mutations, their role in DNA repair and association with PARPi response has been well studied and clearly defined, while for other putative DNA repair defects, more clarification is required before they can be used as therapeutic biomarkers.

EMSY amplification belongs to the latter group, as reports of its role in the HR pathway have been conflicting.[3,4] Here, we describe the prevalence of *EMSY* amplification or overexpression in OC, and summarise the research to date on the function of EMSY, particularly in the context of DNA repair by the HR pathway.

Amplification and overexpression

The *EMSY* gene (also known as *C11orf30*) maps to chr11q13 and is frequently amplified in ovarian and other cancer types, including breast, and head and neck cancer (Fig. 1A).[5] It is reported in around 6-18% of high-grade serous ovarian carcinomas (HGSOC), although it has also been reported at a lower prevalence in other subtypes of OC, such as high-grade endometrioid and clear-cell cancers of the ovary.[1, 3, 6] Two discrete regions within the 11q13 amplicon can be amplified separately or together, depending on the cancer type,[7, 8] and both of these are gene dense, making it challenging to identify genes responsible for driving the amplification (Fig. 1B). The first region includes *CCND1*, *TAOS1/2*, *FADD*, *PPF1A1* and *EMS1*, while the second region includes *EMSY*, *PAK1, RSF1* and *GAB2.* All of these genes have been proposed as potential oncogenes, although *EMSY* and *CCND1* have been identified as the most likely drivers.[3, 7] It is also worth noting that in OC, *EMSY* is often amplified independently of *CCND1*, in around 60% of cases, making it a more likely driver in this cancer type.[3] Interestingly, *EMSY* amplification has not been found to be mutually exclusive

with the common defects in the HR pathway, such as *BRCA1/2* mutations or *BRCA1* methylation. This is in contrast to the mutual exclusivity generally observed for *BRCA1/2* mutations and *BRCA1* methylation in OC, suggesting a strong selection for loss of one of the key members of HR pathway.[1]

EMSY amplification has been associated with poor survival in breast cancer,[3] similarly to *BRCA1/2* mutations;[10] however, this may only be the case when *EMSY* is co-amplified with *CCND1*, at least for ER-positive breast cancer.[11] In OC, *EMSY* amplification and mRNA overexpression have also been reported in association with worse survival.[7, 12]

Amplification of the *EMSY* gene in OC and in other cancer types has been reported to correlate with *EMSY* mRNA expression in multiple studies;[1, 3, 4, 6] while reports on the association between EMSY mRNA and protein expression have been limited and conflicting. Altinisik *et al.* investigated the EMSY gene and protein overexpression in 50 sporadic OC, with mRNA overexpression found in 6 cases (12%). Increased levels of EMSY protein expression were reported in all of these overexpressed cases.[13] In contrast, Wilkerson *et al.* looked at *EMSY* overexpression in 10 *EMSY*-amplified cancer cell lines, including five breast cancer lines and one OC cell line (OVCAR3). While correlation between *EMSY* amplification and mRNA expression was observed ($p = 0.004$), no such observation was made for protein expression. No significant protein expression differences were observed in cell lines with or without amplification. Furthermore, EMSY protein expression did not correlate with Cyclin B1 expression suggesting that EMSY was not expressed in a cell cycle-dependent manner, unlike expression observed for BRCA2, a key member of the HR pathway, which expression is initiated before S-phase, when the HR pathway is active.[14]

Function in DNA repair

The EMSY protein was initially identified through yeast two-hybrid screening with BRCA2, showing that an evolutionarily conserved EMSY N-terminal (ENT) domain binds to the transactivation domain of BRCA2 (N-terminus), and through this interaction EMSY could negatively regulate BRCA2 function in the HR pathway (Fig. 2A-B).[3] Thus, amplification or overexpression of EMSY was suggested to have a similar effect to *BRCA2* inactivating mutations, leading to HR deficiency. In line with its suggested role in the HR pathway, EMSY was shown to co-localise with γH2AX at sites of DNA damage (after irradiation).[3] Furthermore, experiments performed by overexpressing N-terminal EMSY showed that EMSY overexpression could lead to increased chromosomal instability, resulting

in a BRCA2-deficient phenotype with increased replication slippage and reduced HR activity.[15] However, unlike BRCA2 defects, overexpression of N-terminal EMSY resulted in decreased rate of spontaneous HR and normal activity of single-strand annealing pathway.[16]

A major limitation of these early studies into the role of EMSY in DNA repair was that forced expression of partial EMSY (N-terminal) used in the experiments may not equate to the endogenous overexpression of full-length EMSY. In contrast to these studies, Wilkerson *et al.* demonstrated that unlike *BRCA1/2* mutations, endogenous amplification of *EMSY* did not lead to decreased RAD51 (a marker of HR DNA repair) or γH2AX (a marker of DNA damage) foci formation in response to irradiation, platinum or PARPi treatment.[4] Marked reduction in RAD51 foci is the accepted read-out for reduced HR DNA repair activity and as such, EMSY amplification has arguably not caused true experimental HR deficiency. Furthermore, neither mRNA nor protein overexpression were associated with *in vitro* platinum or PARPi (olaparib) sensitivity in the tested cell lines, which included five breast cancer and one OC lines.[4] Although in another study, four OC (three HGSOC – OVCAR3, OV177, OV167 and one clear-cell OC– OVTOKO) cell lines with *EMSY* amplification were reported to have increased PARPi sensitivity (to rucaparib), compared with cell lines without HR pathway alterations.[17]

More recently, Jelinic *et al.* proposed an alternative mechanism of EMSY involvement in the HR pathway (Fig. 2C).[18] By overexpressing different fragments of EMSY (full length, N-terminal and C-terminal), they suggested that since overexpression of C-terminal EMSY resulted in the greatest reduction of HR activity, as measured by DR-GFP reporter assay,[19] and this region of the protein does not interact with BRCA2, it is likely that EMSY's role in the HR pathway is BRCA2-independent.[18] This study also assessed EMSY phosphorylation in the context of HR deficiency. Firstly, phosphorylation of EMSY at S209 by AKT1[20] was unlikely to be linked to HR, as overexpression of full-length EMSY mutated at S209 did not result in changes to HR pathway activity in comparison to a regular overexpression vector. However, mutation of another proposed phosphorylation site, T207, resulted in the stabilization of HR activity to wild-type levels.[18] While this study broadly implicated EMSY in the HR pathway, at best only a moderate reduction (less than 50%) in HR activity was observed, as measured by the DR-GFP assay. Furthermore, this study also failed to observe reduced RAD51 foci formation in an *EMSY*-amplified OC cell line (OVCAR3) in response to DNA damage, and stable knock down of EMSY did not influence the foci formation.

Outside of the involvement in the HR pathway, a number of alternative roles have been proposed for EMSY, most of which still require additional clarification. These include the involvement in chromatin remodelling together with BS69 and HP1β;[3, 21] the negative regulation of interferon-stimulation genes in BRCA2-dependent manner;[20] and EMSY being a possible transcription factor as part of the EMSY/ETS1/KDM5B/miR-31 pathway and the EMSY/KDM5A complex.[22, 23]

In summary, while the early evidence supporting EMSY's involvement in the HR DNA repair pathway was compelling, there remain a number of important discrepancies, including, no clear correlation between *EMSY* mRNA and protein overexpression, and the lack of impact on suppressing RAD51 foci formation (a marker of HR activity) in response to DNA damage in *EMSY*-amplified cells. It is possible that amplification or overexpression of *EMSY* alone may not be enough to induce a full HR deficiency like *BRCA1/2* mutations. More investigation is required to establish the extent of *EMSY*'s involvement in HR, and whether it can be used as a predictive biomarker for platinum and PARPi therapy responses. Future studies should focus on assessing the association between *EMSY* amplification or overexpression and genomic HR-deficiency markers (including mutational and rearrangement signatures, and HRD scores), as well as platinum and PARPi responses. Furthermore, since drug sensitivity assessment performed in 2D cell line models can often be difficult to translate to the clinic, future drug sensitivity studies should be performed in models more closely resembling tumor response in the patient, such as organoids or patient-derived xenografts.

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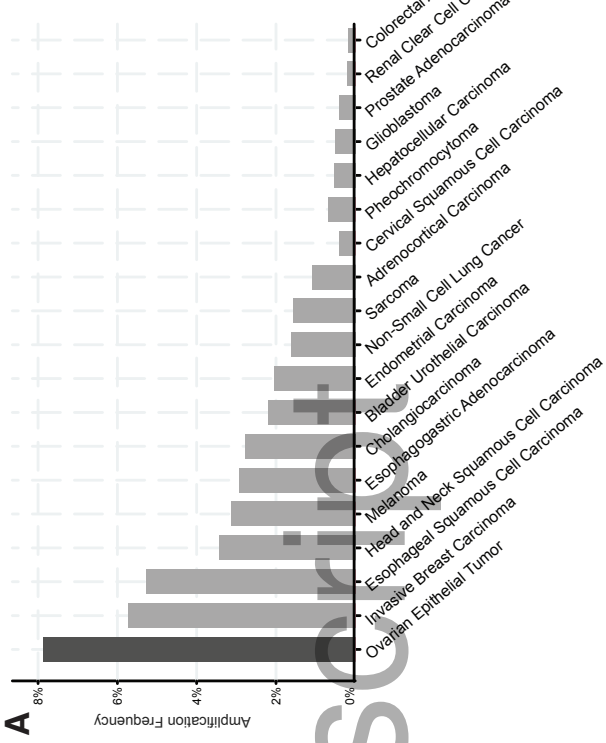
Figure legends

Figure 1. *EMSY* amplification in cancer. (A) *EMSY* amplification rates in ovarian and other cancer types in the The Cancer Genome Atlas PanCancer cohort, n=10528 (generated using cBioPortal[9] on 4th March 2019). (B) Two discrete regions within 11q13 amplicon commonly amplified in ovarian and other cancer types, adapted from Brown *et al.* 2008.[7]

Figure 2. Proposed models of HR deficiency driven by EMSY overexpression. (A) A simplified diagram of HR pathway with normal EMSY expression. (B) A BRCA2-dependent model of HR deficiency driven by EMSY overexpression proposed by Hughes-Stamm *et al.* 2003. (C) A BRCA2-independent model of HR deficiency driven by EMSY overexpression proposed by Janic *et al.* 2017.



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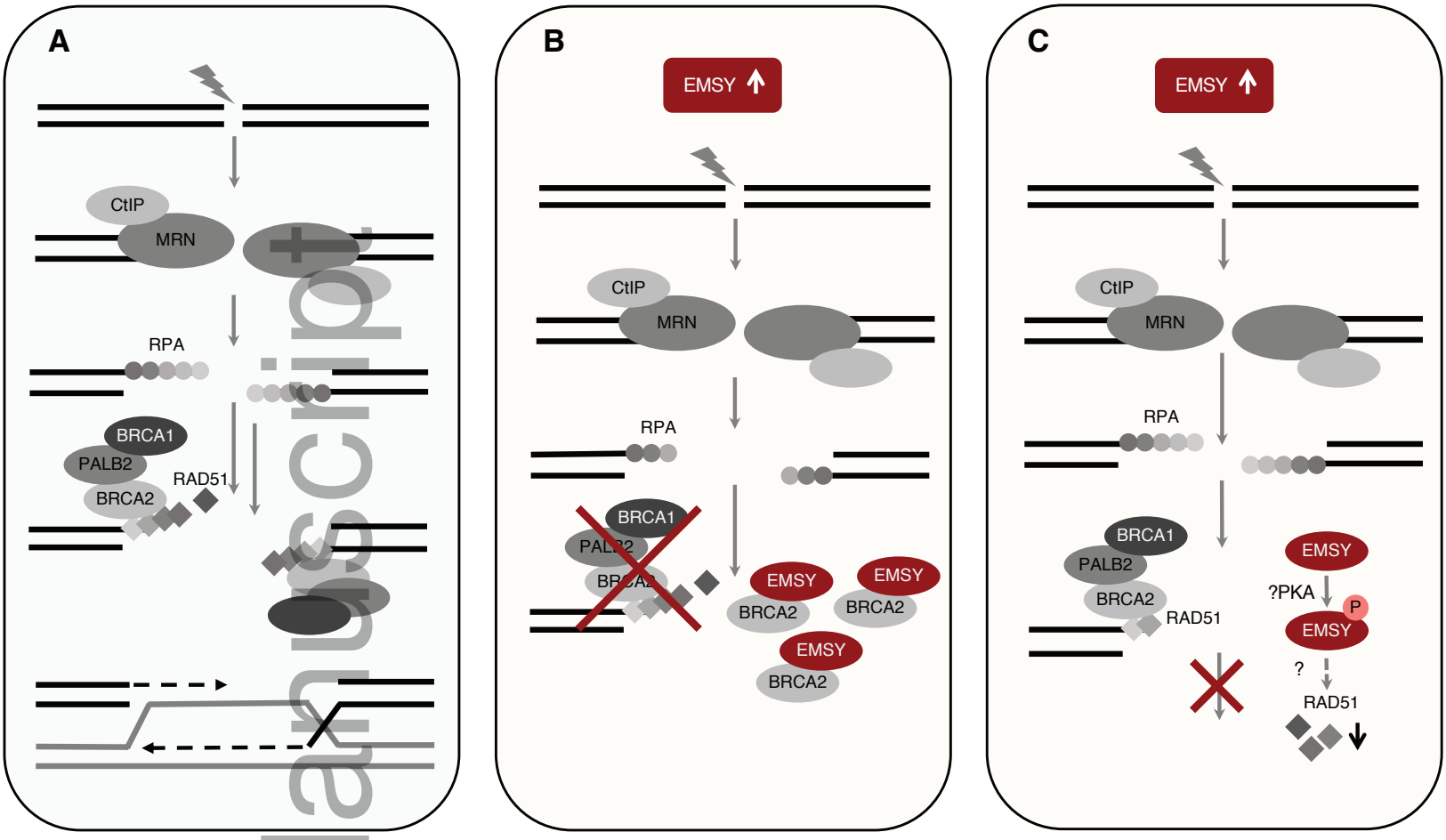

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