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Modulation of Radiation Sensitivity by Targeting Protein Interface

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1. Introduction

Many factors that are involved in DNA damage response determine cellular radiation sensitivity [1]. Such factors' function includes damage signaling, DNA repair, checkpoint, cell cycle regulation and cell death. In molecular terms, protein-protein interaction between the factors or protein-DNA interaction underlies the functions. Therefore modulation of radiation sensitivity and the related cellular functions might be feasible by targeting interfaces between DNA damage response factors (DDRF).

DDRF interfaces are generally defined by long and laborious process of mutagenesis of the factors and by examining its consequent effect on the function. Instead we might employ a simplified strategy of using bioinformatics and DNA/protein analysis tools which are freely available in internet. Here we introduce such programs or tools and demonstrate their usefulness. In addition, we also provide some typical experimental results that validate the use.

2. Methods and Results

Determination of factors that are responsible for radiation sensitivity are identified by utilizing both experimental and theoretical approaches. Generally theoretical side of the investigation involves extensive use of bioinformatics tools. DNA and protein analysis programs are the main tools. Once the computer-based *in silico* analysis is complete, experimental examination ultimately identifies DDRF. Thus *in silico* analysis serves the role as a framework for experimental demonstration. Here are described the procedure to identify radiomodulatory protein interface in the order its use in real laboratory setting.

2.1 Starting material

Since the protein interface assumes at least two protein partners at the beginning, selection of the starting DDRF is essential.

For the purpose, a DDRF is selected first from the literature depending on the cellular function that is to be modulated. Sequence information about the DDRF is obtained from two different sources. One is CGAP web site from National Institute of Health in the United States and the other HPRD from Johns Hopkins University. The address is as follows: http://cgap.nci.nih.gov/, and http://www.hprd.org/. Since these two websites enlist only published information, unnecessary waste of time can be prevented considering many bioinformatics web sites list information that is not experimentally validated.

By typing in the key word or name of a DDRF in the either web site, amino acid sequence of the DDRF is obtained.

2.2Protein sequence analysis

Important domain or motif in the amino acid sequence is shown in HPRD or can be identified by BLAST. For example, one of the DDRF, 53BP1 shows BRCT domains.

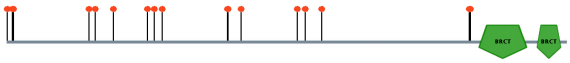

Figure 1. 53BP1 domains by HPRD: Green indicates BRCT domains and red dot attached to a vertical line indicates phosphorylation.

To find domain from BLAST program, copy and pasted 53BP1 sequence into BLASTp program (http://www.ncbi.nlm.nih.gov/).

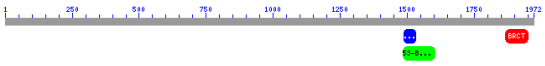

Figure 2. 53BP1 domain from BLAST: found one additional domain, TUDOR (in green) domain in addition to BRCT (in red).

Since the functional significance of the domains is already known in protein-protein interaction (BRCT domain) or in protein-DNA interaction (TUDOR domain), any proteins that interacts with the protein domains are putative candidate for a modulator of radiation sensitivity. Or chemicals that are able to modify the domain's protein interaction or that change three dimensional protein conformation are potential radiosensitivity-modifying agents.

2.3 Finding interface for macromolecules interaction and binding partner

From the HPRD web site, known binding partners can be found. In such a case, binding interface can become the domain. Or potential candidates are inferred from the analysis using ELM web site (http://elm.eu.org). In that case, binding interface is the specific motif that implies potential binding. For example, it shows possible domains that are necessary for interaction or modification such phosphorylation.

Such information enables predicting putative candidates for interaction. One of the ELM analyses of 53BP1 is shown.

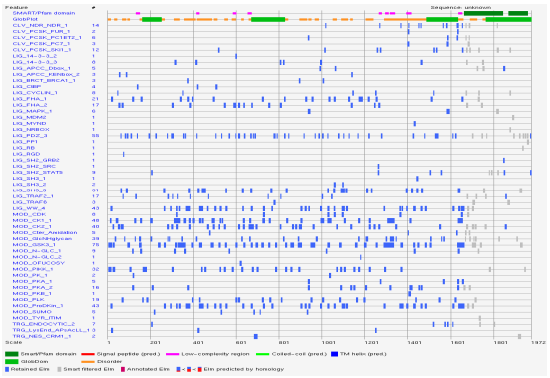

Figure 3. ELM analysis of 53BP1. Blue bars indicate motifs. Several of them are necessary for interaction and suggest potential binding partner.

2.4 Finding interface for macromolecules interaction and binding partner by experimental approaches

Since the prediction based on computer analysis is seldom appreciated as an ultimate solution, experimental data should be supplemented. To find protein binding partner to the domain, yeast two hybrid (Y2H) approach can be used to isolate potential candidate. Once potential binding protein is identified, further confirmation of the interaction is required as the Y2H shows many false positives.
As an alternative approach, general protein-binding chaperones such as heat shock proteins could be investigated for potential modulator.

2.5 Evidence for modulation of radiation sensitivity

From the aforementioned analyses, DDRF, DDRF-interacting protein, molecular chaperone or chemicals that change those proteins' activities are potential modulator for radiation sensitivity.

For example BRCT domain-interacting gamma-H2AX peptide has been demonstrated as a modulator [2]. Or chaperone inhibitor has been shown as a modulator (Figure 4). By inhibiting chaperone function, the chaperone cannot maintain DDRF conformation or stability. This in turn results in radiosensitivity. In our case, we used chemical X that is able to mimic Hsp90 chaperone inhibitor.

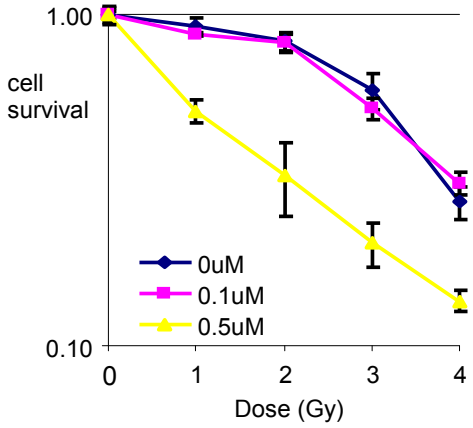

Figure 4. Chaperone inhibitor X enhances cellular radiation sensitivity.

3. Conclusion

We described two different approaches that are useful to study modulator of radiation sensitivity. By combining computer based in silico analysis and experimental data, rational design for identification of radiomodulator could be facilitated. This strategy should aid further screening of chemical modulator and ultimately radiotherapy.

Acknowledgement

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