様式３

神戸大学バイオシグナル総合研究センター共同利用研究報告書

平成 31 年 3 月 25 日

神戸大学バイオシグナル総合研究センター長 殿

所属機関・部局名 名古屋大学・環境医学研究所・発生遺伝分野
職 名 研究員
研究代表者名 郭朝万

下記のとおり平成30年度の共同利用研究成果を報告します。

記

（課題番号:301003）

1. 共同利用研究課題名	Investigating the molecular mechanism of transcription coupled nucleotide excision repair initiation				
2. 共同利用研究目的	Transcription coupled NER (TC-NER) is a dedicated DNA repair system that specifically removes cytotoxic lesions that block gene transcription, however the mechanism of TC-NER initiation remains poorly understood. In this joint research proposal, we will use a live-cell imaging approach of endogenous GFP-tagged TC-NER factors in combination with local UV-C irradiation to understand how TC-NER factors cooperates during the initiation step of TC-NER.				
3. 共同利用研究期間	平成 30 年 4 月 1 日 ～ 平成 31 年 3 月 31 日				
4. 共同利用研究組織					
氏 名	所属部局等		職名等	役 割 分 担	
(研究代表者) 郭 朝万	名古屋大学・環境医学研究所・発生遺伝分野		研究員	研究総括・研究実施	
(分担研究者) 荻 朋男 賈 楠	同上 同上		教授 特任助教	研究総括・研究協力 研究協力	
5. センター内受入研究者	研究部門・分野名	シグナル統合経路研究部門・ゲノム機能制御研究分野	氏 名	菅澤 薫 教授	

※ 次の6～9 の項目は，枠幅を自由に変更できます。但し，6～9 の項目全体では1頁に収めて下さい。

（課題番号：301003）

6. 共同利用研究計画

Nucleotide excision repair (NER) is a critical repair system that removes a various types of DNA lesions. NER contains two sub-pathways: Global Genome NER (GG-NER) repairs DNA lesions generated throughout the whole genome, while TC-NER specifically repairs the DNA damages on actively transcribed genes. In comparison to the well-studied GG-NER, the molecular mechanisms of TC-NER, especially the initiation step of TC-NER, remains largely unclear for more than 20 years. Since GG-NER is responsible for approximately 90% of the total repair executed by NER, to obtain a better understanding of the molecular basis of TC-NER initiation, we planned to generate a XPC knock out cell lines using CRISPR/Cas9 technique, which are defective for GG-NER. Then we also planned to generate a cell line library based on the generated XPC-KO cell lines, in which each core TC-NER factors (e.g. UVSSA, CSA/CSB, USP7) is knocked out, respectively. We will further generate a set of cell lines with endogenously expression of GFP-tagged NER-factors (e.g. XPA, XPB et al.). By using these cells and the developed living cell imaging techniques in Biosignal Research Center, Kobe University. We will study how TC-NER factors are involved in repair of UV induced DNA damage. Comparison of the recruitment kinetics of general NER factor will give us valuable information on the reaction order, spatiotemporal organization and the kinetic framework of TC-NER.

7. 共同利用研究の成果

In the past year, we had successfully generated XPC-KO cells using CRISPR/Cas9 technique, and the GG-NER deficiency in this cell lines was confirmed by UDS assay; moreover, we performed a special UDS assay (TSA-UDS), of which combined UDS assay with the tyramide-based signal amplification, to measure the TC-NER activity in GG-NER deficient cells. Since we mainly aim to obtain a better understanding of the molecular mechanism of TC-NER initiation, thus a cell line library based on the generated XPC-KO cell lines, in which each core TC-NER factors were sequentially knocked out, were successfully generated and the defects of TC-NER activity in these cell lines were confirmed by Recovery of RNA synthesis assay. The critical part of this joint research was to study the behavior of fluorescent-tagged NER proteins after UV damage in the absence of each TC-NER factors. We set out to generate GFP-NER-factor knock-in cell lines, but unfortunately these works is still under ongoing due to the low efficiency of gene editing. If this approach is not successful, the library of cell lines stably expressing GFP-tagged NER factors or transiently transfection method will be considered. In future, we will use these cell lines for live imaging studies.

8. 共同利用研究成果の学会発表・研究論文発表状況

（本センターの担当教員の氏名の記載，又はこの共同利用研究に基づくとの記載のある論文等を記載して下さい。なお，論文の場合は，別刷りを1部提出してください。）

Not applicable.

9. 共同利用研究に関連した受賞，博士学位論文の取得，大型研究プロジェクトや競争的資金の獲得等がありましたらご記入ください。

Not applicable.