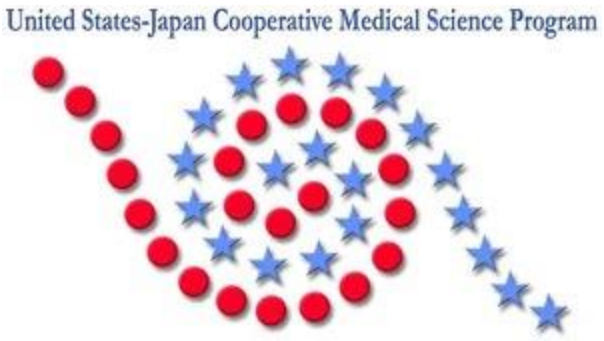

The Joint 24th International Conference on Emerging Infectious Diseases in the Pacific Rim of the U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)

ABSTRACT E-BOOK

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SESSION ONE: EBV and Gastric Cancer and Lymphoid Malignancies

Atsushi Kaneda – Chiba University

EBV rewires 3D chromatin and epigenetically drives gastric tumorigenesis

Suk Kyeong Lee – The Catholic University of Korea

Epstein-Barr Virus Disrupts Sphingolipid Metabolism to Maintain Viral Latency in Gastric Carcinoma

Teru Kanda – Tohoku Medical and Pharmaceutical University

Epstein-Barr virus strains transmitted among Transeurasian language speakers

Over 95% of adult humans worldwide are infected with the Epstein–Barr virus (EBV). EBV is transmitted from human to human via saliva, implying that people with the same ethnic origin are likely to be infected with similar EBV strains. In Asian countries, EBV-associated nasopharyngeal carcinomas (NPC) are more common in southern China and Southeast Asia. In comparison, the incidence of chronic active EBV infection (CAEBV) is higher in Japan and Korea. Since EBV genome sequences of Korean and Japanese EBV sequences were limited, we determined the entire viral sequences of two Korean EBV strains derived from gastric cancer cell lines (SNU-719 and YCCEL1) and seven Japanese EBV strains derived from tonsillar tissues (1,2). Phylogenetic analyses of our Korean and Japanese-derived EBV sequences and previously published EBV genome sequences from the database show that, among Asian EBVs, Japanese and Korean EBVs constitute an independent subgroup, distinct from the EBV strains derived from the NPC-endemic region (i.e., southern China). A recent study indicates that ancient people living in the West Liao River area spread east and west to become present Transeurasian language speakers (Japanese, Korean, Mongolian, and others) (3). We are currently testing the hypothesis that Transeurasian language speakers harbor similar EBV strains distinct from Chinese strains.

Yu Uemura – St. Marianna University School of Medicine

Advances in pathology and treatment of chronic active Epstein-Barr virus Disease

Chronic active EB virus disease (CAEBV) is a disease in which T or NK cells are infected with EBV and clonally proliferate. It causes systemic and persistent inflammatory symptoms such as fever, liver damage, uveitis, vasculitis, and skin lesions. As it progresses, hemophagocytic syndrome develops which can be fatal. This rare disease is often diagnosed originally as a of fever of unknown origin or liver damage, but by the time when it is appropriately diagnosed as CAEBV, it may have progressed to be more serious. The main symptoms are inflammation, but some cases progress to T/NK cell lymphoma and follow a fatal course. Previously, cases were reported mainly from Japan, South Korea and other East Asian countries. However, since the disease was listed as a type of EBV-positive T, NK cell lymphoproliferative tumor in the WHO classification published in 2017, the number of reports has increased globally. CAEBV is broadly classified into systemic type, called systemic CAEBV (sCAEBV), and cutaneous type. Cutaneous type is further divided into two types: hydroa vacciniforme-like lymphoproliferative disorder and severe mosquito bite allergy. The results of a nationwide survey on sCAEBV conducted in Japan showed that the median age of onset is 21, and half of the cases occur in adults.

In most cases, EBV infects B cells using CD21 as an infection receptor, but it has been reported that T cells and NK cells also express CD21 and can be infected by EBV. The mechanism of the tumorigenesis of EBV-infected cells is speculated that there are factors on both the virus side and the host side. Inflammatory symptoms are thought to be caused by inflammatory cytokines such as IFN-γ and TNFα produced by EBV -infected cells or cells surrounding them.

sCAEBV cannot be cured by chemotherapy, and allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment. Furthermore, it has been reported that the cases with active disease at the time of HSCT result in poor prognosis. Therefore, disease control before HSCT may contribute to improve treatment outcomes. Regimens containing steroids and cell-killing antitumor drugs are used to control disease activity before transplantation, but the optimal treatment method has not been determined yet. We reported that STAT3 is constitutively activated in sCAEBV-infected cells and contributes to the immortalization of infected cells and the production of inflammatory cytokines. We then evaluated the therapeutic effects of ruxolitinib, a JAK1/2 inhibitor that inhibits STAT3. CR rate and OR rate after eight weeks or at early termination were 22.2% and 33.3%, respectively. No one showed progressive disease. The results indicate that ruxolitinib demonstrates encouraging effects and is a potent therapeutic reagent against sCAEBV with active disease.

To further improve prognosis of sCAEBV, we need to identify optimal biomarkers and to develop treatment methods. We are currently identifying cytokines that reflect disease status and become biomarkers. We are also investigating to develop an optimal conditioning regimen and a donor source of allogeneic transplantation. Antiviral therapy for sCAEBV is also an attractive option in the future.

Hisashi Iizasa – Shimane University

Inhibitors of viral microRNA promote Epstein-Barr virus-associated cancer cell death

[Introduction] Epstein-Barr virus (EBV), a member of the Gammaherpesvirus subfamily, infects lymphocytes and epithelial cells, and persistently EBV-infected cells develop EBV-associated cancers, such as B-lymphoma, nasopharyngeal carcinoma (NPC) and EBV-associated gastric cancer. EBV is characterized by encoding up to 40 microRNAs (BART miRNAs) in its genome, which suppress host cell apoptosis and evade immune recognition mechanisms in EBV-associated cancers. We therefore hypothesized that BART miRNAs might be potential targets for antiviral agents. However, the BART miRNA promoter has not been extensively analyzed. In this study, we searched for compounds that inhibit transcription of the BART miRNA promoter and induce apoptosis in cells.

[Methods] The DNA of the BART miRNA promoter region was cloned upstream of the luciferase gene. Deletion mutants and point mutations of the promoter region were generated and DNA sequences important for promoter activity were identified. The secreting NanoLuc gene was inserted downstream of the wild-type promoter, and NanoLuc activity was measured after addition of drugs that suppress BART miRNA promoter activity. EBV-infected and -uninfected tumor cells were treated with the identified drugs, and the inhibitory effect on cell proliferation specific to EBV-associated tumor cells was confirmed.

[Results] A region critical for BART promoter activity was identified. Within the identified region, a single nucleotide polymorphism was found that is frequently observed in EBV strains from NPC-affected areas, was found. Using point mutant plasmids, we identified ETS family binding sites that enhance transcription. And reduced BART promoter activity was observed in ETS-A-deficient cell lines. From 1,200 FDA-approved drugs, we found that the HDAC inhibitor Vorinostat and the Src family kinase inhibitor Dasatinib suppress BART promoter activity. These two drugs inhibited BART gene transcription and increased apoptosis in EBV-positive epithelial cells. Treatment of EBV-positive epithelial cells with Vorinostat increased the expression of apoptosis-inducing proteins targeted by BART miRNAs, resulting in apoptosis of the treated cells.

[Conclusion] BART miRNAs suppress the expression of apoptosis-promoting factors, innate immunity genes, and acquired immunity-related genes. Therefore, a decrease in BART miRNA expression may inhibit the progression of EBV-associated epithelial malignancies.

SESSION TWO: HPV and Cancer

Iwao Kukimoto – National Institute of Infectious Diseases

Intra-Patient Variation of Human Papillomavirus Genome Contributing to Cervical Cancer Development

Human papillomavirus (HPV) is the causative agent of cervical cancer with a circular DNA genome of approximately 8000 base pairs and is known to immortalize cells through the function of viral oncoproteins E6/E7. However, the action of E6/E7 alone does not generate invasive cancer. For infected cells to acquire malignant traits, the cell genome must accumulate mutations and chromosomal aberrations over a decade of persistent HPV infection. Recent integrative cancer genomics studies have shown that genomes of several cancers, including cervical cancer, accumulate somatic mutations (C-to-T and C-to-G substitutions) characteristic of cellular APOBEC3 cytosine deaminase. It has also been shown that E6/E7 upregulate APOBEC3B expression, suggesting that APOBEC3 is an important host factor linking HPV infection and cervical cancer development.

We explored intra-patient nucleotide sequence variation in HPV genomes in cervical lesion specimens using next-generation sequencing and found that HPV genomes from low-grade cervical lesions frequently contain C-to-T substitutions in TpC dinucleotides, a mutation signature caused by APOBEC3. On the other hand, high-level, non-synonymous substitutions other than APOBEC3-type mutations were often detected in the viral E1/E2 genes of cervical cancer specimens. Since E1/E2 play important roles in viral genome replication, transcription, and maintenance, their mutations may be positively selected for the development of HPV-related cancer. In this presentation, we report a comprehensive profile of APOBEC3 and non-APOBEC3 mutations detected in the HPV genome of clinical specimens and discuss their potential roles in cervical cancer development.

Ayumi Taguchi – Osaka University

Carcinogenesis of HPV18-associated cervical cancer

Human papillomavirus 18 (HPV18) is a highly malignant HPV genotype among high-risk HPVs, characterized by the difficulty of detecting it in precancerous lesions and its high prevalence in adenocarcinomas. Its carcinogenesis and cellular targets remain unclear.

To elucidate its carcinogenesis, we conducted the spatial multi-omics analysis of four cervical cancers with mixed histological types. Phylogenetic analysis based on the whole exome analysis revealed that the different histological types had a common cell of origin in each case. Moreover, the HPV-derived transcriptome and HPV integration sites were common among different histological types. In addition, Human gene expression profiles indicated that HPV18-positive cancer retained immunologically cold components with stem cell properties.

Subsequently, to identify its cellular targets, we established HPV18 long control region regulated GFP-expressing lentiviral vector (HPV18LCR-GFP). HPV18LCR-GFP vectors were transduced into patient-derived squamocolumnar junction organoids and Single-cell RNA sequencing of GFP-positive and GFP-negative cells was conducted. Among upregulated genes in GFP-positive cells, NPM3 most significantly suppressed HPV18 LCR activity. Furthermore, NPM3 knockdown of HPV18-infected cells downregulated stem cell-related genes.

It is suggested that the carcinogenesis of HPV18 mixed carcinoma may be associated with the maintenance of a stem cell component. In addition, target cells of HPV18 are undifferentiated, and NPM3 was associated with its replication. From these results, the maintenance of undifferentiated cells might be essential for the HPV18 carcinogenesis.

Tomomi Nakahara – National Cancer Center Research Institute

In vitro carcinogenesis model of HPV- induced cervical adenocarcinoma

Cervical cancer is the second most common cancer for women under 39 years old with approximately 11,000 new cases and 2,900 death each year in Japan. Squamous cell carcinoma (SCC) and adenocarcinoma (ADC) are two major histological subtypes accounting for approximately 75% and 23% of cervical cancers, respectively. The total incidence of cervical cancer has declined in many high-income countries mainly due to the decline of SCC. However, the relative and absolute rates of ADC have instead raised over a few decades with increased prevalence particularly among young women. In Japan, the incidence of cervical cancer has been increased in the last two decades with the higher

prevalence of ADC under 39 years old in compared to older age groups. Many studies associate ADC with a worse prognosis than SCC. However, it is not well understood the molecular mechanisms of carcinogenesis of HPV induced cervical ADC. Therefore, we need a better understanding of the carcinogenesis of ADC to develop effective screening and/or treatment.

To gain better insights into molecular background of ADC, we aimed to establish an in vitro carcinogenesis model of ADC. We previously reported the establishment of an in vitro model for cervical squamous cell carcinoma by introducing defined viral and cellular oncogenes, HPV16 E6 and E7, c-MYC and activated RAS to human cervical keratinocytes. In this study, the expression or knockdown of potential lineage-specifying factors was introduced in addition to the defined four oncogenes to direct carcinogenesis toward ADC and the cell properties associated with the cell lineage were analyzed in monolayer and organoid cultures and the tumors in mouse xenografts. Our results suggested that FOXA2 plays a vital role in dictating the histopathology of cervical cancers (Zhang M, et al., Cancer Sci. 2022). With these findings, we would like to discuss a potential model of HPV induced ADC.

Kayo Togawa – National Cancer Center

Japan's progress and challenges with achieving the global targets to eliminate cervical cancer

Given the high global burden of cervical cancer for which effective preventive measures exist, the World Health Organization (WHO) launched the Cervical Cancer Elimination Initiative (CCEI) in 2020 with a strategy to eliminate cervical cancer as a public health problem worldwide. To eliminate cervical cancer, all countries should achieve the so-called 90:70:90 targets by 2030: 1) 90% of girls are fully vaccinated with HPV vaccine by the age of 15 years; 2) 70% of women are screened with a high-performance test by the age of 35 years and again by the age of 45 years; and 3) 90% of women identified with cervical disease receive treatment. As a result of CCEI, a growing number of countries have shown their commitment to reach the scale-up goals and have made progress towards elimination of cervical cancer. Although Japan got off to a slow start and lags behind other high-income countries like Australia, Japan has also made progress, e.g., resumption of the nationwide vaccination program for girls and addition of the nonavalent vaccine with two-dose schedule (for girls who receive the first dose by the age of 15 years) to the program. In my presentation, I will speak about Japan's progress and challenges with achieving the global targets with the focus on HPV vaccination and cervical screening and also discuss strategies to improve cervical cancer prevention in Japan.