Prognostic impact of DNA ploidy and protein expression of enhancer of zeste homologue 2 (EZH2) in synovial sarcoma

PhD thesis

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I. Introduction

Synovial sarcoma is an aggressive soft tissue tumor with possibly mesenchymal stem cell origin. It is also a well-known "translocation-associated tumor" with characteristic t(X;18) (p11.2;q11.2), represented in more than 95% of the cases. *SYT* functions as a transcription coactivator containing an SNH domain which interacts with SWI/SNF ATPase-associated chromatin remodeling complex and transcription factors such as AF10 whereas *SSX* acts as a transcription corepressor containing an SSXRD which interacts with polycomb group. The SYT-SSX fusion protein, which retains almost the entire SYT including SNH with losing the last 8 amino acids at the N-terminal side and replaced by the last 78 amino acids of SSX containing SSXRD. By activating and silencing of the target genes epigenetically, the fusion protein causes general alteration of cellular programming. The prognosis varies between 36 to 76% of 5-year survival rate and the prognostic factors (size, age, mitoses and poorly-differentiated areas) are also poorly defined indicating the need of identifying new markers.

Controversies exist among the ploidy, karyotype regarding the prognosis; on the other hand, EZH2 is a core member of the polycomb repressing complex 2 (PRC2) which functions as a histone methyltransferase by producing a characteristic H3K27me3 epigenetic mark. It plays an important role in cell cycle regulation, DNA damage repair, cell differentiation, senescence, and apoptosis. EZH2 overexpression has been found in many tumors including carcinomas and lymphomas and it is associated with aggressive clinical behavior. However, EZH2 in soft tissue sarcomas are rarely been discussed and its expression profile and the clinical relevance in particularly in synovial sarcomas, are still largely unknown.

II. Objective

My work was divided in two parts; the first part was to establish the correlation among the ploidy, karyotypic complexity regarding the clinical outcome. The second part was to investigate the relevance of EZH2 in synovial sarcomas. I summarized as follow

1. To investigate the DNA ploidy using image cytometry with fine-tune interpretation and correlates the result with high-resolution comparative genomic hybridization (HR-CGH) and clinical outcome.
2. To investigate the utility of EZH2 as a diagnostic marker in synovial sarcomas by comparing its expression (both in mRNA and protein levels) cross the histological subtypes, the molecular features and clinical data.
3. To elucidate the functional correlation between EZH2 and its epigenetic marker, H3K27me3.
4. To investigate the impact of EZH2 expression, along with H3K27me3 and Ki-67, on overall survival based on Kaplan-Meier curve.

III. Materials and Methods

Fifty-five primary tumors without preoperative chemoradiotherapy were selected including 6 poorly differentiated (PDSS)-, 39 monophasic (MPSS)-, and 10 biphasic (BPSS) - synovial sarcomas, respectively in FFPE state. 9 cases fresh frozen samples containing 2 PDSS, 4 MPSS and 3 BPSS were also available for HR-CGH analysis. Image cytometry was performed with the help of Feulgen stain and histogram was generated after measuring 30 reference- and 200 tumor- cells, respectively. Aneuploidy was identified when abnormal stem line was found, whereas the complex- or simple-diploidy was defined by the presence or absence of cells harbored "5c-exceeding events". Meanwhile, HR-CGH was also performed on the selected 9 cases and both positive and negative controls were also used. The result of ploidy, karyotype and the clinical outcome were correlated.

Tissue microarrays were created from those 55 cases and immunostained with EZH2, H3K27me3 and Ki-67 and evaluated by pre-established scoring criteria. Results of the three immunostainings were compared, and differences were sought between the histological subtypes as well as patient groups defined by gender, age, tumor size, tumor location, the presence of distant metastasis, and the type of fusion gene. Quantitative real-time PCR was also performed to detect the EZH2 expression in mRNA level as well to correlate the protein level. The relationship between EZH2 expression and survival was plotted on a Kaplan-Meier curve.

IV. Results

IV.1 Significant differences among aneuploid, simple diploid and complex diploid groups

Ten-, 12- and 33- cases of aneuploidy-, complex diploid- and simple diploid-group were identified by image cytometry, respectively.

In HR-CGH analysis; the aneuploid group contained a large number of genetic alterations with the sum gain of at least 2 chromosomes; the complex diploid group showed substantial but less aberrations whereas the simple diploid one, the chromosomal aberrations were hardly detectable.

Eighty percent, 50% and 39% of aneuploidy-, complex diploid- and simple diploid- group developed metastasis, respectively. By using Fisher's exact test the three groups proved to be significantly different.

IV.2 High expression of EZH2 and high abundance of H3K27me3 in PDSS

High expression of EZH2 mRNA and protein levels was specifically detected in the PDSS. EZH2 scores were found to correlate with those of Ki-67 and H3K27me3 indicating high EZH2 expression is associated with higher mitotic activity and the functional participation of PRC2, respectively.

IV.3 EZH2 as a potential prognostic marker in synovial sarcoma

Cross all subtypes; cases with high EZH2 score were characterized by larger tumor size (≥ 5cm), distant metastasis, and poor prognosis. Such association hold true without PDSS implying in the MPSS and BPSS; higher expression of EZH2 was associated with higher proliferation rate, larger tumor size, and the risk of developing early distant

metastasis. In these histological groups, EZH2 was superior to Ki-67 in predicting tumor growth rate and distant metastasis.

V. Discussion

Majority of synovial sarcomas, irrespective of the histological subtype, exhibit simple karyotypes additional to its specific t(X;18) translocation. Secondary genetic anomalies are sometimes seen, but these alterations are often variable and inconsistent. Measurement of the total DNA content gains high importance in estimating the prognosis of the disease in terms of the selection of the aggressiveness of the therapy. In the first part of my work; we were able to separate diploid synovial sarcoma in to complex diploid and simple diploid groups, respectively, which reflect distinct, karyotypic and prognostic features. Our result of image cytometry showed correlations with HR-CGH and the prognosis and it is regarded as fast, easy to perform and inexpensive tool which can be used as a screening method in diagnosed synovial sarcomas.

On the other hand; although the pathomechanism in synovial sarcomas which lead to EZH2 up-regulation still need to be elucidated, the possible mechanism may due to *Myc*-associated up-regulation, induced by HIF1α and fusion protein or down-regulation of microRNAs. The overexpressed EZH2 may silence several target genes participating in apoptosis, mitosis, tumor-suppressing and angiogenesis which may lead to abnormal proliferation and aggressive behavior; it is important to establish the correlation between EZH2 and H3K27me3 since not all the tumors which show overexpressed EZH2 are always associated with its epigenetic mark. The reason may due to general down-regulation of PRC2-target genes, the formation of tumor-specific PRC2 which may have different substrate specificity, and also phosphorylation-induced conformation change, etc. High EZH2 status, in our investigation, was found to be predictive of fast tumor growth and distant metastasis in the MPSS+BPSS group which may explain the

variable clinical outcome even in the better differentiated synovial sarcomas. Thus, while not sufficiently specific when applied alone, EZH2 can be used as an auxiliary immunohistochemical marker of the poorly differentiated subtype in doubtful cases (e.g., better-differentiated histomorphology coupled with high mitotic rate, or vice versa). Moreover, EZH2 status, along with other our previously finding, the prognostic impact of ploidy, may refine the current stratification of MPSS and BPSS patients into low- and high-risk subgroups, thus influencing prognosis and possibly also the therapeutic strategies.

VI. Conclusions

Our investigations showed consistent association between DNA ploidy, karyotypic complexity and also the clinical outcome. Study the epigenetic deregulation opens a new insight of oncogenesis of tumors; we are the first group investigating EZH2 expression profile and the clinical relevance of synovial sarcomas. We summarized our results as following:

1. Complex diploid group is associated with complex karyotype based on “single cell aneuploidy” phenomenon and has worse prognosis than simple diploid group.
2. EZH2 can serve as diagnostic adjunct since its expression helps to distinguish PDSS from the MPSS and BPSS. Its overexpression is also associated with unfavorable clinical outcome.
3. EZH2 expression correlates with H3K27me3 indicating functional participation of PRC2.
4. High EZH2 expression can be a predictive marker in terms of fast tumor growth rate and early development of metastasis which are particularly useful in MPSS and BPSS.
5. Both DNA ploidy and EZH2 expression have valuable prognostic impact in synovial sarcomas, since they offer complement data for clinicians in terms of patient management. EZH2 can also be the target for the epigenetic therapy, especially in combination with other epigenetic modulators and conventional chemotherapy to achieve maximized and synergetic effect.

VII. Publication records

Publications related to the theme

1. Changchien YC, Tátrai P, Papp G, Sápi J, Fónyad L, Szendrői M, Pápai Z, Sápi Z. (2012) Poorly differentiated synovial sarcoma is associated with high expression of enhancer of zeste homologue 2 (EZH2). J Transl Med, Oct 30;10:216.
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2. Balogh Z, Szemlaky Z, Szendroi M, Antal I, Pápai Z, Fónyad L, Papp G, Changchien YC, Sápi Z. (2011) Correlation between DNA ploidy, metaphase high-resolution comparative genomic hybridization results and clinical outcome of synovial sarcoma. Diagn Pathol, Nov 3;6:107.
IF:1,64

3. Changchien YC, Katalin U, Fillinger J, Fónyad L, Papp G, Salamon F, Sápi Z. (2012) A challenging case of metastatic intra-abdominal synovial sarcoma with unusual immunophenotype and its differential diagnosis. Case Rep Pathol, 2012:786083.

Publications not related to the theme

1. Papp G, Changchien YC, Péterfia B, Pecsenka L, Krausz T, Stricker TP, Khoor A, Donner L, Sápi Z. (2013) SMARCB1 protein and mRNA loss is not caused by promoter and histone hypermethylation in epithelioid sarcoma. Mod Pathol, Mar;26(3):393-403.
IF: 4,792

2. Changchien YC, Haltrich I, Micsik T, Kiss E, Fónyad L, Papp G, Sápi Z. (2012) Gonadoblastoma: Case report of two young patients with isochromosome 12p found in the dysgerminoma overgrowth component in one case. Pathol Res Pract, Oct 15;208(10):628-32.
IF: 1,213

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