A COMPARISON OF RT-PCR AND FISH TECHNIQUES IN MOLECULAR DIAGNOSIS OF EWING'S SARCOMA IN PARAFFIN-EMBEDDED TISSUE

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Summary

Ewing's sarcoma is relatively uncommon tumor representing 6-8 percent of malignant bone tumors with variable morphology. Cytogenetically, Ewing's sarcomas are characterized by a specific reciprocal chromosomal translocation t(11;22)(q24;q12). The presence of this chromosomal translocation has been detected in approximately 85 percent of the cases. The translocation results in the fusion of EWS gene from chromosome 22 to FLI1 gene at 11q24 which is a member of ETS family of transcription factors. In this study we performed a comparison of two molecular diagnostic strategies, namely RT-PCR and FISH, in fresh, frozen and formalin-fixed paraffin-embedded tissues. We conclude that FISH is a more sensitive technique than RT-PCR for the diagnosis of Ewing's tumors in formalin-fixed paraffin-embedded tissue. In conclusion, molecular pathology techniques, using reverse transcription-polymerase chain reaction (RT-PCR) and/or fluorescence in situ hybridization (FISH) are valuable diagnostic tools for evaluation of undifferentiated small round-cell tumors like Ewing's sarcoma.

Key words: Ewing's sarcoma – chromosomal translocation – RT-PCR – FISH

Souhrn

Molekulární diagnostika Ewingova sarkomu: porovnání RT-PCR a FISH metod pro tkáně zalité do parafinu

Ewingův sarkom je relativně vzácný nádor reprezentující 6–8 procent nádorů kostí. Cytogeneticky je Ewingův sarkom v 85 procentech případů charakterizován specifickou recipročí chromozomální translokací t(11;22)(q24;q12), která má za následek fúzi genu EWS na chromozomu 22 a genu FLI1 na chromozomu 11. V této studii jsme se zaměřili na porovnání dvou molekulárně diagnostických metod – reverzně transkripční polymerázové řetězové reakce (RT-PCR) a fluorescenční in situ hybridizace (FISH). Z našich výsledků vyplývá, že v případě formalínem fixované, do parafinu zalité tkáně je patrně vlivem degradace RNA, FISH senzitivnější než RT-PCR. Závěrem: molekulárně patologické metody RT-PCR a FISH jsou účinným diagnostickým nástrojem pro diagnostiku nádorů Ewingova typu.

Klíčová slova: Ewingův sarkom – chromozomální translokace – RT-PCR – FISH

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Ewing's sarcoma is a relatively uncommon tumor representing 6-8 percent of malignant bone tumors. However, it is the second most common sarcoma in bone and soft tissue in children (22). Ewing's sarcoma and primitive neuroectodermal tumors (PNET) are defined as round cell sarcomas that show varying degrees of neuroectodermal differentiation (14).

In the Ewing's sarcoma, in contrast to PNET, features of neuroectodermal differentiation are lacking as assessed by light microscopy and immunohistochemistry. The primitive round to oval Ewing's sarcoma cells contain in their cytoplasm glycogen aggregates and produce fine cytoplasmic processes with primitive intercellular function (13). No specific immunohistochemical marker of this tumor exists till now.

Cytogenetically, Ewing's sarcomas are characterized by a specific reciprocal chromosomal translocation t(11;22)(q24;q12). The presence of this chromosomal translocation has been detected in approximately 85 percent of the cases (15, 17). Subsequent cloning of the translocation breakpoint showed (24), that chromosomal translocation t(11;22)(q24;q12) results in the fusion of Ewing sarcoma breakpoint region 1 gene (EWS) from chromosome 22 to Friend leukemia virus integration 1 gene (FLI1) at 11q24 which is a member of ETS (v-ets erythroblastosis virus E26 oncogene homolog) family of transcription factors (4, 18). Moreover, another chromosomal translocation t(11;22)(q22;q12) was found in 10-15 percent of cases, which results in the expression of EWS-ERG fusion transcript. In 1 % or less cases t(7;22), t(17;22), and t(2;22) translocations and inv(22) have been described (4, 9, 12, 19, 21). The mentioned secondary chromosomal aberration resulted in fusion between EWS gene and one of the ETS superfamily: Ets variant gene 1 (ETV1), Ets variant gene 4 (E1AF), fifth Ewing variant gene (FEV), and zinc finger sarcoma gene (ZSG), respectively.

Little is known about the function of the genes involved in this translocation. EWS gene encodes an ubiquitously expressed RNA binding protein of an unknown function. EWS was found to be uniformly expressed in two splicing variants of similar abundancy, EWS a and EWS b, which differ in a single amino acid (11). The EWS protein, primarily localized in the nucleus, has been found to associate with components of the basal transcriptional machinery (2,16, 23) and RNA splicing factors (10, 23), as well as with partition into the ribosome-dense fraction of the cytoplasm, in particular, upon G protein coupled receptor signaling (5).

All ETS members are defined by the 87 amino acid domain that is both necessary and sufficient for the site-specific DNA-binding in vitro (6). ETS factors are thought to act by binding to promoter and/or enhancer elements of the target genes and result in the transcriptional activation or repression.

In this study we performed a comparison of two molecular