UNIVERZITA PALACKÉHO V OLOMOUCI
LÉKAŘSKÁ FAKULTA
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V Olomouci 31. srpna 2015

Zápis o konání obhajoby disertační práce ve studijním programu Patologická anatomie a soudní lékařství

Mariam Gachechiladze, MD vědecká pracovnice Ústavu klinické a molekulární patologie LF UP a studentka prezenční formy doktorského studijního programu *Patologická anatomie a soudní lékařství* LF UP v Olomouci.

Téma disertační práce: **„Molecular markers of drug resistant non-small-cell lung cancer."**

Obhajoba se konala v Olomouci 31. srpna 2015 v 11.00 hodin.

Komise:

předseda: prof. MUDr. Zdeněk Kolář, CSc. .....

místopředseda: prof. MUDr. Jiří Ehrmann, Ph.D. .....

členové: doc. MUDr. Svetlana Brychtová, Ph.D. .....

doc. MUDr. Martin Tichý, CSc. .....

doc. MUDr. Jaroslav Horáček, CSc. .....

doc. MUDr. Leoš Křen, Ph.D. .....

Oponenti: doc. MUDr. Leoš Křen, Ph.D. .....
Ústav patologie LF MU a FN Brno

doc. MUDr. Radovan Matěj, Ph.D. .....
Ústav patologie 3. LF UK Praha

Školitel: MUDr. MVDr. Jozef Šrarda, Ph.D. .....

Předsedající přednesl stručnou charakteristiku uchazeče, hodnocení školitele. Poté uchazeč vyložil podstatný obsah své disertace. Oponenti přednesli své posudky. Uchazeč odpověděl na připomínky a dotazy oponentů. Ve vědecké rozpravě vystoupili: viz příloha – zápis o diskusi.

Hlasování se účastnilo .....6..... členů komise. Kladně hlasovalo .....6..... členů, záporně .....0..... členů, neplatných lístků bylo odevzdáno .....0..... .

Usnesení:
Přítomní členové komise tajným hlasováním rozhodli, že **Mariam Gachechiladze, MD** obhájila disertační práci a doporučili udělení akademického titulu doktor ve zkratce Ph.D. dle § 47 Zákona o vysokých školách č. 111/98 Sb.

.....
prof. MUDr. Zdeněk Kolář, CSc.
předseda komise

Leos Kren, Assoc. Prof., M.D., PhD

Question: Did you have any cases with borderline immunohistochemical reactivity? If yes, how did you solve this/these cases?

Answer: In case of Filamin A expression there has been mainly four patterns: weak, weak to moderate, moderate and strong and in preliminary analysis I evaluated it as 1+, 2+, 3+, 4+ multiplied with percentage, resulting final histoscore 400. However, the evaluation with standard histoscore method as weak (1+), moderate (previous 2+ and 3+) and strong (previous 4+) multiplied with percentage of positive tumor cells, with final histoscore 300 showed the same results, so it has been used for final analysis of data.

doc. MUDr. Radoslav Matěj, Ph.D

Question: Poněkud méně obvyklá antagonistická exprese BRCAl u pacientů v rozdílných stádiích pokročilosti NSCLC byla již diskutována v dizertační práci, nicméně spíše obecně. Má aspirantka nějakou teorii, proč tomu tak je? Je známanějaká asociace s patogenními somatickými mutacemi či germináinímy mutacemi genu BRCA1?

Answer: Expression of BRCA1 and its inverse relationship with patient outcome in early and advanced NSCLC seems to be related to yet unknown underline factors.Even in literature we did not find explenation for this fenomenon. We did not find an independent prognostic value of BRCA1 protein immunohistochemistry in multivariate age adjusted analysis. Interestingly, BRCA1 expression was significantly correlated with increased patient age. Usually, it is known that there are no BRCA1 mutations in lung cancer patients. However, some recent studies show that in 4% of squamous cell and 6% of adenocarcinomas of lung show BRCA1 mutation.

Question: V jakém ohledu vidí aspirantka možnost dalšího využittí expresního profilu zvolených markeru u pacientů s NSCLC?

Answer: An usefulness of **BRCA1** immunohistochemistry in clinical practice doesn't seem to be promising prognostic or predictive marker in case of NSCLC patients;

RAD51 can be a better surrogate marker for DNA damage repair capacity and immunohistochemistry seems to be more promising. Our findings suggest that immunohistochemical expression of RAD51 may serve as simple and powerful prognostic and predictive biomarker in patients with NSCLC. The present data justify further validation of RAD51 immunohistochemistry in larger cohorts of NSCLC patients.

Filamin A may represent an important prognostic marker for NSCLC progression and might help to predict platinum-based treatment resistance. Also, filamin A might be used as a therapeutic target to sensitise cells to DNA damaging chemotherapy;

Our data suggest that **S1P lyase** quantification might represent a useful companion marker for NSCLC patients together with SphK1. Further investigations are requested to evaluate the role of S1P lyase in cell culture models in order to provide further evidence of this correlation in lung cancer.

Question: Nezkoumala aspřantka expresi vybraných markeru taky jinou metodikou (např. na úrovni mRNA)?

Answer: The aim of the present study was to evaluate an **immunohistochemical expression** of selected **proteins** in relationship to patient **survival. The study the expression of mRNA and protein relationship is our further aim.**

Question: Byly výsledky studie rovněž korelovány se standardně vyšetřovanýni prediktivními markery u NSCLC? A pokud ano, pak s jakým výsledkem?

Answer: We did not correlate the status of expression of the above mentioned proteins with mutation status of EGFR and expression of ALK. We used a retrospective patient setup where EGFR status and ALK expression was not known.

prof. MUDr. Zdeněk Kolář, CSc.

Question: Why did not you study the mRNA expressio of the selected markers.

Answer: There was no sufficient funding for this kind of molecular testing.

Question: Did you calculate the cut-off values for marker expression?

Answer: In case of BRCA1 protein expression I used generally accepted cut-off value of 10%. The cut-off value for RAD51 expression was calculated by online software "cut-off" finder, from Charite Medical University, Berlin and cut-off values for SphK1, SPL and Filamin A expression was calculated by statistician using Cox-proportional hazards regression model.

doc. MUDr. Svetlana Brychtová, Ph.D.

Question: did you try to compare the differences in marker expression between different histological types?

Answer: yes, I did comparisons in all available clinicopathological groups, including histological subtypes. Only statistically significant difference I've found was the highest expression of sphingosine-1 phosphate lyase in adenocarcinomas and sphingosine-1 phosphate kinase expression in large cell carcinomas.

prof. MUDr. Jiří Ehrmann, Ph.D.

Question: Which antibody did you use for SphK1 staining and how was it validated?

Answer: I used "in house" antibody, which was provided by our collaborator from Institut de Pharmacologie et de Biologie Structurale, Toulouse, France and was validated at the same institute. The main reason why we chose to study SphK1 immunohistochemical expression from the whole sphingolipid metabolism pathway proteins is that the antibody against this protein is best validated.