University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

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2. Supervisor:

   Surname: Roseweir Forename: Antonia

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3. Research Project Report

3.1 Project Title

Effect of Src/Fyn inflammatory pathway on survival in colorectal cancer patients

3.2 Project Lay Summary

Colorectal cancer (CRC) is the second most common cause of cancer death in Europe, with survival rates only 50%. Current therapies for CRC are not optimal and new targets are needed to help better treat CRC. Stimulation of inflammation within the tumour itself could be a novel target as this inflammation is associated with improved patient survival. However, the pathways modulating inflammation within the tumour are not yet established. We therefore aim to investigate one inflammatory pathway called the Fyn pathway within 758 CRC patient tumour samples, to assess if this pathway affects inflammation or is associated with patient survival.

3.3 Start Date: 1st August 2016 Finish Date: 31st August 2016

3.4 Original project aims and objectives

We aim to investigate the association between the local inflammatory infiltrate – Fyn and cancer specific survival for a 758 colorectal cancer patient cohort. We will characterise the impact of an active Fyn pathway on clinicopathological characteristics of these patients, to ascertain how the pathway is influencing tumour progression. This will be achieved using immunohistochemistry to stain and score individual nuclear Fyn within a 758 patient sample tissue microarray, with full anonymized follow up data. We will then analyse the data for patient survival, clinicopathological characteristics and inflammatory mediators.

3.5 Methodology

I carried out immunohistochemistry to stain a 758 colorectal cancer patient tissue microarray for FYN, firstly defining the parameters needed. I also scored the stained sections using the weighted histoscore method. This was double scored by Dr Roseweir.

I analysed the data using SPSS to investigate the effect of Fyn downstream pathway activation score on cancer specific survival. This data will then be used to investigate associations with inflammation and clinicopathological characteristics.

Finally, western blots were carried out to investigate the presence of SFK416, Fyn, and pSTAT3 so as to confirm the correlation found in the data analysis with the two colorectal cancer cell lines HT29 and T84 from the previously collected lysates. I also carried out some cell culture to prepare a fresh batch of lysates because of unforeseen problem with the previously collected lysates.

3.6 Results

1. Association with cancer-specific survival

   We found out that there is an association between expression level of activated nuclear Fyn with cancer-specific survival when nuclear Fyn is analysed together with SFK416 (P=0.047), as seen in Table 1. However individually, expression level of neither nuclear Fyn nor SFK416 is associated with cancer-specific survival. As shown in Figure 1, high expression of inactive Fyn decreases cancer-specific survival by almost 1 year in a study of 444 colorectal cancer patients.

2. Association with clinicopathological characteristics and inflammation

   As seen in Table 2, level of individual expression of SFK416 is shown to be a significant independent biomarker for tumour proliferation (P=0.049). Table 2 also shows that high expression of nuclear FYN is associated with tumour site (P=0.021), BRAF status (P<0.001), molecular subtype (P=0.002), tumour proliferation (P=0.006), tumour necrosis (P=0.04), and tumour stroma percentage (P=0.025). Nuclear Fyn is also associated with level of inflammation indicated by the Glasgow Microenvironment Score (GMS) (P=0.037) as seen in Table 2. Moreover, expression level of activated SFK416 and Fyn correlates with BRAF status (P<0.001), tumour proliferation (P=0.004), and tumour necrosis (P=0.023) as shown in Table 2. However, on multivariate analysis in Table 3, there is no statistically significant correlation between SFK416 and nuclear Fyn and cancer-specific survival rate.

3. Western Blot Analysis

The western blot of Dasatinib-treated HT29 and T84 colorectal cancer cells reveals the presence of SFK416, Fyn, and pStat3 only on HT29 – BRAF+ve as seen in Figure 1. This confirms the associations of Fyn expression with BRAF+ve mutation.

3.7 Discussion

This study investigated the effects of Fyn in colorectal cancer patients. Assessment of both SFK416 and Fyn together, shows that expression of activated nuclear Fyn improves colorectal cancer-specific survival. This is possibly due to significant correlation of high expression of active Fyn with high tumour proliferation, low tumour necrosis level as seen in Table 3 (P=0.004, P=0.023). It is known that high tumour proliferation is a key for effective treatment for cancer (1). Therefore, this may increase cancer-specific survival. On the other hand, high tumour necrosis has been shown to further stimulate host inflammatory responses, which eventually promotes tumour growth (2). Hence it results in poorer prognosis of patients undergoing colorectal cancer resection (2).

Improved colorectal cancer-specific survival in patients with high expression of nuclear Fyn may be because of the correlation between high Fyn expression with lower inflammation level measured in GMS. Decreasing GMS is associated with increasing survival in colorectal cancer (3). In addition, cancer specific survival is also correlated with the different molecular subtypes. Those with high Microsatellite Instability immunity, lower tumour stroma percentage, and high Ki67 confer a higher chance of survival, which were found increasingly so in patients with high Fyn expression. However, patients with low Fyn expression have KRAS mutation, high tumour stroma percentage, and low Ki67 and thus, tend to have poorer prognosis.

Other studies on other types of cancer such as prostate cancer have shown that overexpressed Fyn attenuates morphologic transformation into malignancy through activating downward signalling pathway of PAX and FAK (4). This is contrary to what is seen in this colorectal cancer study cohort where high level of Fyn correlates with higher

cancer-specific survival. While another study on clear cell renal carcinoma showed that Fyn is associated with good prognosis (5). One possible reason is that in different types of cancers, Fyn may activate different molecules in its downward signalling pathway such as pSTAT3. Thus, further study looking into the effects of a specific downward Fyn signalling pathway such as pSTAT3 on colorectal cancer will be worthwhile. Unfortunately, due to time constraint, we were unable to complete our analysis of pSTAT3.

Therefore, this study shows that inhibition of Fyn does not present as a one-size-fits-all therapeutic solution for cancer. Inhibition of a more specific molecule downstream of Fyn pathway will presumably have higher specificity in treating a specific type of cancer.

Significant correlation of Fyn with BRAF+ve seen in patients' data was confirmed by the western blotting done on the Dasatinib treated HT29 cell lines. We found that Fyn is only expressed in BRAF+ve mutated HT29 cells. Dasatinib is shown to completely inhibit phosphorylation at Src416 site. pStat3 is also shown to be inhibited by Dasatinib in a dose dependant manner.

In conclusion, expression of Fyn together with SFK416 is still not statistically significant to be a prognostic marker for colorectal cancer survival and a further research looking into pSTAT3 downward signalling molecule should be considered.

4. Reflection

I am grateful for this valuable experience, which allowed me to discover more about experimental therapeutics in cancer science. It gave me an insight into the research world and introduced me to a variety of wet lab skills such as western blotting, immunohistochemistry staining, and cell culture. On top of that, I also had the chance to perform data analysis and interpretation of the lab result using SPSS, which allowed me to find a relationship between my lab results and the clinicopathology of colorectal cancer.

Having to do these protocols a couple of times honed my dexterity, perseverance, and accuracy. These traits are of paramount importance in the research world. Working with unexpected results produced by various protocols trained us to be flexible and also anticipative. I am so glad to participate in this research programme as I was given the chance to work independently after being given clear instructions, which gave me a real taste of working in a lab and a sense of responsibility to the result that will be produced.

Prior to this experience, I always had the idea that research is dry and boring. I was proven wrong, clinical research could be very interesting and rewarding. The unpredictable nature of research itself forces researchers to be persistent, forward-looking, and flexible. I learned that trial and error and failures are common in research area so it is tremendously rewarding to finally be able to relate our findings to the clinical cases. This experience has encouraged me to incorporate research in my future medical career with a first step of undertaking an intercalated degree after my third year.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

6. Signatures:

Supervisor	Date	Student	Date
	01/09/16		31/08/16

References:

1. Tennant DA, Durán RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. Nature Reviews Cancer. 2010 Mar 19;10(4):267–77.

2. Richards CH, Roxburgh CSD, Anderson JH, McKee RF, Foulis AK, Horgan PG, McMillan DC. Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer. British Journal of Surgery. 2011 Nov 16;99(2):287–94.

3. Park JH, McMillan DC, Powell AG, Richards CH, Horgan PG, Edwards J, Roxburgh CSD. Evaluation of a tumor Microenvironment-Based Prognostic score in primary operable Colorectal cancer. Clinical Cancer Research. 2014 Dec 3;21(4):882–8.

4. Saito YD, Jensen AR, Salgia R, Posadas EM. Fyn: a novel molecular target in prostate cancer. Cancer. 2010 Apr 1;116(7):1629–37.

5. Roseweir AK, Qayyum T, Lim Z, Hammond R, MacDonald AI, Fraser S, Oades GM, Aitchison M, Jones RJ, Edwards J. Nuclear expression of Lyn, a Src family kinase member, is associated with poor prognosis in renal cancer patients. BMC Cancer. 2016 Mar 16;16(1).

Appendices:

	Nuclear		
	N (%)	10yr-CSS % (SE)	*P*
SFK^{416} (n=404)			
Low expression	291 (72)	78 (3)	0.467
High expression	113 (28)	82 (5)	
FYN (n=466)			
Low expression	111 (24)	80 (5)	0.355
High expression	355 (76)	79 (2)	
SFK^{416} + FYN (n=444)			
Low expression	84 (19)	85 (5)	**0.047**
Moderate expression	268 (60)	75 (3)	
High expression	92 (21)	86 (4)	

Table 1: Relationship between SFK and Fyn expression and cancer specific survival in patients undergoing elective, potentially curative resection of colorectal cancer.

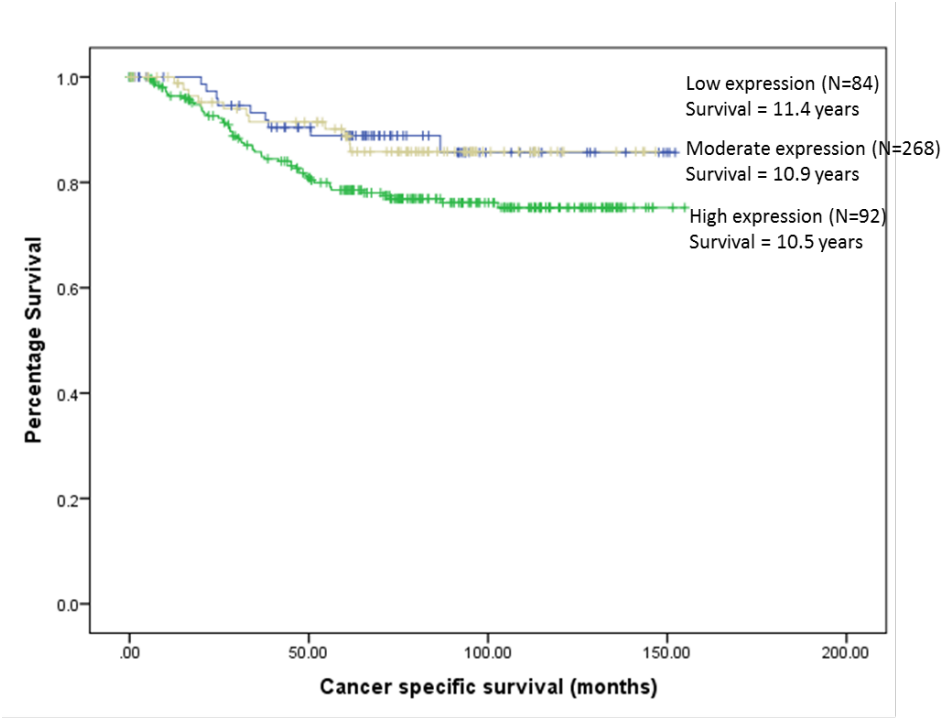

Figure 1: Relationship between expression of nSFK416/nFyn and cancer specific survival.

	n416			nFYN			n416+FYN			
	Low (n=328)	High (n=117)	*P*	Low (n=111)	High (n=356)	*P*	Low (n=84)	Mod (n=269)	High (n=92)	*P*
Age			0.608			0.566				0.587
<75	176 (54)	66 (56)		63 (57)	191 (54)		48 (57)	141 (52)	53 (58)	
>75	152 (46)	51 (44)		48 (43)	165 (46)		36 (43)	128 (48)	39 (42)	
Sex			0.742			0.633				0.393
Female	160 (49)	55 (47)		52 (47)	176 (49)		37 (44)	137 (51)	41 (45)	
Male	168 (51)	62 (53)		59 (53)	180 (51)		47 (56)	132 (49)	51 (55)	
Tumour site			0.777			**0.021**				0.104
Colon (right-side)	143 (44)	54 (46)		39 (35)	171 (48)		31 (37)	119 (44)	47 (51)	
Colon (left-side)	104 (32)	34 (29)		39 (35)	105 (29)		32 (38)	79 (29)	27 (29)	
Rectum	81 (24)	29 (25)		33 (30)	80 (23)		21 (25)	71 (27)	18 (20)	
BRAF status			0.089			**<0.001**				**<0.001**
Wild type	266 (82)	87 (74)		103 (94)	267 (75)		78 (93)	212 (79)	63 (68)	
Mutant	59 (18)	30 (26)		6 (6)	87 (25)		5 (6)	55 (21)	29 (32)	
Molecular Subtype			0.830			**0.002**				0.063
MSI Immune	108 (33)	37 (32)		25 (23)	128 (36)		21 (25)	91 (34)	33 (36)	
Low TSP/High Ki67	87 (27)	36 (31)		28 (25)	96 (27)		22 (26)	71 (26)	30 (33)	
KRAS mutant	68 (21)	18 (15)		25 (23)	71 (20)		19 (23)	53 (20)	14 (15)	
High TSP/Low Ki67	44 (13)	17 (15)		27 (24)	36 (10)		18 (21)	35 (13)	8 (9)	
Other	20 (6)	9 (7)		6 (5)	23 (7)		4 (5)	18 (7)	7 (7)	
T-stage			0.524			0.791				0.592
1	22 (7)	7 (6)		6 (5)	24 (7)		4 (5)	20 (7)	5 (5)	
2	61 (19)	19 (16)		23 (21)	59 (17)		21 (25)	42 (16)	17 (19)	
3	175 (53)	64 (55)		58 (52)	193 (54)		43 (51)	145 (54)	51 (55)	
4	70 (21)	27 (23)		24 (22)	80 (22)		16 (19)	62 (23)	19 (21)	
Differentiation			0.726			0.883				0.862
Mod/well	298 (91)	105 (90)		100 (90)	319 (90)		76 (90)	245 (91)	82 (89)	
Poor	30 (9)	12 (10)		11 (10)	37 (10)		8 (10)	24 (9)	10 (11)	
Vascular invasion			0.521			0.051				0.740
Absent	228 (70)	85 (73)		86 (77)	242 (68)		61 (73)	190 (71)	62 (67)	
Present	100 (30)	32 (27)		25 (23)	114 (32)		23 (27)	79 (29)	30 (33)	
Margin involvement			0.127			0.225				0.156
No	317 (97)	109 (93)		108 (97)	337 (95)		83 (99)	257 (96)	86 (93)	
Yes	11 (3)	8 (7)		3 (3)	19 (5)		1 (1)	12 (4)	6 (7)	
Peritoneal involvement			0.647			0.601				0.966
No	259 (79)	90 (77)		85 (77)	281 (79)		66 (79)	210 (78)	73 (79)	
Yes	69 (21)	27 (23)		26 (23)	75 (21)		18 (21)	59 (22)	19 (21)	
Mismatch repair status			0.071			0.267				0.161
Competent	286 (88)	92 (81)		96 (88)	295 (84)		77 (92)	227 (85)	74 (82)	
Deficient	40 (12)	22 (19)		13 (12)	57 (16)		7 (8)	39 (15)	16 (18)	
Microsatellite Instability			0.532			0.095				0.346
MSS	277 (89)	92 (87)		99 (92)	282 (86)		77 (93)	221 (88)	71 (87)	
MSI	34 (11)	14 (13)		9 (8)	47 (14)		6 (7)	31 (12)	11 (13)	
Proliferation			**0.049**			**0.006**				**0.004**
Low	152 (46)	42 (36)		62 (56)	146 (41)		46 (55)	120 (45)	28 (30)	
High	176 (54)	75 (64)		49 (44)	210 (59)		38 (45)	149 (55)	64 (70)	
Necrosis			0.314			**0.040**				**0.023**
Low	191 (60)	75 (66)		60 (55)	224 (65)		49 (59)	151 (59)	66 (74)	
High	125 (40)	39 (44)		50 (45)	118 (35)		34 (41)	107 (41)	23 (26)	
Tumour stroma percentage			0.204			**0.025**				0.775
Low	260 (79)	86 (74)		78 (70)	287 (81)		63 (75)	210 (78)	73 (79)	
High	68 (21)	31 (26)		33 (30)	69 (19)		21 (25)	59 (22)	19 (21)	
Klintrup-Makinen grade	196 (62)	70 (61)	0.907			0.058				0.234
Low	120 (38)	44 (39)		76 (68)	202 (57)		83 (81)	154 (60)	54 (61)	
High				34 (31)	140 (39)		25 (19)	104 (40)	35 (39)	
GMS			0.632			**0.037**				0.105
0	117 (37)	45 (39)		35 (32)	137 (40)		25 (30)	102 (40)	35 (39)	
1	151 (48)	53 (46)		53 (48)	162 (47)		42 (51)	118 (46)	44 (49)	
2	48 (15)	16 (14)		22 (20)	43 (13)		16 (19)	38 (14)	10 (12)	
Crohns-like Reaction			0.334			0.381				0.170
No	278 (88)	104 (91)		94 (88)	303 (89)		71 (86)	229 (89)	82 (92)	
Yes	38 (12)	10 (9)		13 (12)	39 (11)		12 (14)	29 (11)	7 (8)	
mGPS			0.154			0.224				0.093
0	173 (66)	48 (58)		48 (68)	179 (61)		42 (72)	137 (63)	42 (59)	
1	60 (23)	21 (25)		16 (23)	73 (25)		11 (19)	53 (24)	17 (24)	
2	31 (11)	14 (17)		7 (9)	43 (14)		5 (9)	28 (13)	12 (17)	
NLR			0.176			0.605				0.409
Low	240 (82)	71 (76)		62 (78)	262 (80)		53 (79)	196 (82)	62 (76)	
High	53 (18)	23 (24)		18 (22)	65 (20)		14 (21)	42 (18)	20 (24)	

*Total may not equal to 100% as some are rounded off to the nearest whole numbers
mGPS – modified Glasgow Prognostic Scores
NLR – Neutrophils to Lymphocytes Ratio

Table 2: Relationship between SFK and Fyn expression, clinicopathological characteristics and inflammatory responses in patients undergoing elective, potentially curative resection of colorectal cancer.

	All patient (n=405)			
	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Clinicopathological Characteristics				
Age (<75/>75)	1.22 (0.75-1.99)	0.421	-	-
Sex (Female/Male)	1.19 (0.73-1.94)	0.482	-	-
Tumour Site (Colon (right)/colon (left)/Rectum)	0.91 (0.67-1.23)	0.529	-	-
BRAF status (wild-type/mutant)	0.94 (0.52-1.70)	0.835	-	-
Molecular Subtypes (1/2/3/4/5)	1.61 (1.36-1.9)	**<0.001**		**<0.001**
T-Stage (1/2/3/4)	2.05 (1.43-2.95)	**<0.001**		0.914
Differentiation (Moderate or well/Poor)	2.06 (1.02-4.17)	**0.044**		0.256
Vascular Invasion (Absent/Present)	2.26 (1.39-3.69)	**0.001**		**0.003**
Margin Involvement (No/Yes)	4.82 (2.19-10.57	**<0.001**		**<0.001**
Peritoneal Involvement (No/Yes)	3.10 (1.89-5.10)	**<0.001**		**0.002**
Mismatch Repair Status (Competent/Deficient)	0.28 (0.09-0.88)	**0.030**		0.881
Microsatellite Instability (MSS/MSI)	0.34 (0.13-0.92)	**0.033**		0.063
Necrosis (Low/High)	0.95 (0.57-1.59)	0.857	-	-
Ki67 proliferation index (low/high)	0.52 (0..-0.82)	**0.005**		0.293
Tumour Stroma Percentage (<50%/>50%, n=182)	2.10 (1.26-3.51)	**0.005**		**0.020**
Inflammatory Characteristics				
Klintrup-Makinen Grade (Strong/Weak)	0.26 (0.13-0.51)	**<0.001**		0.862
GMS (0/1/2, n=186)	2.43 (1.70-3.47)	**<0.001**		0.139
Crohns-like reaction (no/yes)	0.22 (0.05-0.88)	**0.033**		0.081
mGPS (0/1/2)	1.31 (0.89-1.92)	0.167	-	-
NLR (low/high)	0.70 (0.33-1.47)	0.343	-	-
SFKs				
Nuclear SFK^{416}	0.73 (0.42-1.27)	0.269	-	-
Nuclear FYN	1.30 (0.74-2.29)	0.356	-	-
Nuclear 416+FYN	1.00 (0.70-1.43)	**0.047**		0.613

Table 3: Clinicopathological characteristics of patients undergoing elective, potentially curative resection of colorectal cancer and survival (n=405).

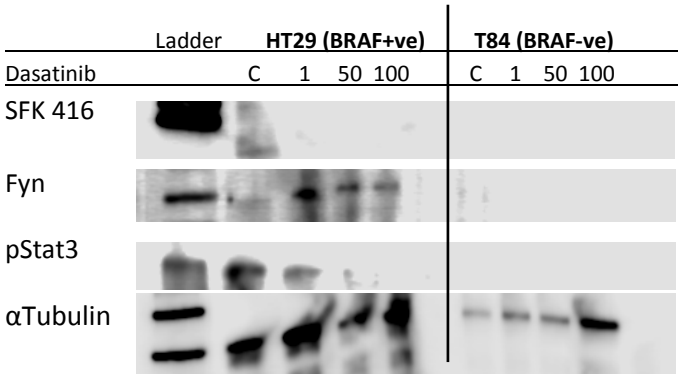

Figure 2: Western blot analyses of SFK416, Fyn, pStat3, and α Tubulin – a control.