



UNIVERSITE DE TLEMCEEN N° D'ORDRE



République Algérienne Démocratique et Populaire

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

UNIVERSITE de TLEMCEEN

Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers

Département de Biologie

Laboratoire de Biologie Moléculaire Appliquée et d'Immunologie

MEMOIRE

Présenté par

DJEBRI Nihel Chahinez

En vue de l'obtention du

Diplôme de MASTER

En Immunologie

Thème

Effet de la metformine sur la prolifération et la cytotoxicité au cours du cancer du sein

Soutenu le 09 juillet 2017, devant le jury composé de :

Président	SMAHI Mohammed Chemseddine	MCA
Examinatrice	BRAHAMI Nabila	MCB
Examineur	GHEZZAZ Kamel	MMA
Encadreur	ARIBI Mourad	Professeur

09/07/2017

Introduction

Le cancer du sein est une maladie hétérogène touchant environ 1.3 million de femmes chaque année à travers le monde (Jemal et al., 2011), c'est une prolifération excessive et incontrôlée causée par des transformations des cellules ou des mutations au niveau des gènes tels que les oncogènes ou les gènes suppresseurs de tumeur (Elenbaas, 2001). Ces cellules acquièrent des caractéristiques qui favorisent la progression et la prolifération tumorale (Hanahan and Weinberg, 2011).

Le système immunitaire peut jouer un double rôle dans la prévention et le développement des tumeurs d'où la théorie d'immunoediting qui implique plusieurs types cellulaires y compris les monocytes (Lakshmi Narendra et al., 2013).

Les monocytes sont une population très hétérogène qui représente 5 à 10% des leucocytes, ils se développent dans la moelle osseuse et peuvent migrer aux tissus en réponse à une inflammation par le mécanisme de chimiotactisme où ils se transforment en macrophages ou en cellules dendritiques (Gordon and Taylor, 2005). Les monocytes peuvent contribuer à la défense immunitaire ou à la progression des tumeurs (Andrea Doseff and Arti Parihar, 2012).

Il existe plusieurs immunomodulateurs qui peuvent avoir un effet antitumorigénique tels que la metformine qui est à la base un médicament antidiabétique oral et qui a été cité dans plusieurs études comme étant une drogue complexe responsable de l'activation de multiples voies impliquées dans l'inhibition de la progression tumorale et l'arrêt de la prolifération cellulaire par ses effets directs et indirects sur les tumeurs.

Dans cette étude on va étudier l'effet de la metformine sur la prolifération des cellules cancéreuses du sein en co-culture avec les monocytes autologues.

Références bibliographiques :

Alizart, M., Saunus, J., Cummings, M., Lakhani, S.R., 2012. Molecular classification of breast carcinoma. *Diagn. Histopathol.* 18, 97–103. doi:10.1016/j.mpdhp.2011.12.003

Allavena, P., Sica, A., Solinas, G., Porta, C., Mantovani, A., 2008. The inflammatory micro-environment in tumor progression: The role of tumor-associated macrophages. *Crit. Rev. Oncol. Hematol.* 66, 1–9. doi:10.1016/j.critrevonc.2007.07.004

Andrea Doseff, Arti Parihar, 2012. *Monocyte Subsets and Their Role in Tumor Progression.* INTECH Open Access Publisher.

Ben-Baruch, A., 2002. Host microenvironment in breast cancer development: Inflammatory cells, cytokines and chemokines in breast cancer progression: reciprocal tumor–microenvironment interactions. *Breast Cancer Res.* 5. doi:10.1186/bcr554

Bhati, R., Patterson, C., Livasy, C.A., Fan, C., Ketelsen, D., Hu, Z., Reynolds, E., Tanner, C., Moore, D.T., Gabrielli, F., Perou, C.M., Klauber-DeMore, N., 2008. Molecular Characterization of Human Breast Tumor Vascular Cells. *Am. J. Pathol.* 172, 1381–1390. doi:10.2353/ajpath.2008.070988

Bhatia, A., Kumar, Y., 2011. Cancer-Immune Equilibrium: Questions Unanswered. *Cancer Microenviron.* 4, 209–217. doi:10.1007/s12307-011-0065-8

Bingle, L., Brown, N.J., Lewis, C.E., 2002. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J. Pathol.* 196, 254–265. doi:10.1002/path.1027

Brempeles, K.J., Crispe, I.N., 2016. Infiltrating monocytes in liver injury and repair. *Clin. Transl. Immunol.* 5, e113. doi:10.1038/cti.2016.62

Cassetta, L., Pollard, J.W., 2016. Cancer immunosurveillance: role of patrolling monocytes. *Cell Res.* 26, 3–4. doi:10.1038/cr.2015.144

Cazzaniga, M., Bonanni, B., Guerrieri-Gonzaga, A., Decensi, A., 2009. Is it Time to Test Metformin in Breast Cancer Clinical Trials? *Cancer Epidemiol. Biomarkers Prev.* 18, 701–705. doi:10.1158/1055-9965.EPI-08-0871

Chan, F.K.-M., Moriwaki, K., De Rosa, M.J., 2013. Detection of Necrosis by Release of Lactate Dehydrogenase Activity, in: Snow, A.L., Lenardo, M.J. (Eds.), *Immune Homeostasis.* Humana Press, Totowa, NJ, pp. 65–70. doi:10.1007/978-1-62703-290-2_7

Clark, G.J., Der, C.J., 1995. Aberrant function of the Ras signal transduction pathway in human breast cancer. *Breast Cancer Res. Treat.* 35, 133–144. doi:10.1007/BF00694753

Clauser, P., Marino, M.A., Baltzer, P.A.T., Bazzocchi, M., Zuiani, C., 2016. Management of atypical lobular hyperplasia, atypical ductal hyperplasia, and lobular carcinoma in situ. *Expert Rev. Anticancer Ther.* 16, 335–346. doi:10.1586/14737140.2016.1143362

Coronella, J.A., Spier, C., Welch, M., Trevor, K.T., Stopeck, A.T., Villar, H., Hersh, E.M., 2002. Antigen-Driven Oligoclonal Expansion of Tumor-Infiltrating B Cells in Infiltrating Ductal Carcinoma of the Breast. *J. Immunol.* 169, 1829–1836. doi:10.4049/jimmunol.169.4.1829

Chapitre 6. Références bibliographiques

- De Kleer, I., Willems, F., Lambrecht, B., Goriely, S., 2014. Ontogeny of Myeloid Cells. *Front. Immunol.* 5. doi:10.3389/fimmu.2014.00423
- Del Barco, S., Vazquez-Martin, A., Cuf?, S., Oliveras-Ferraros, C., Bosch-Barrera, J., Joven, J., Martin-Castillo, B., Menendez, J.A., 2011. Metformin: Multi-faceted protection against cancer. *Oncotarget* 2, 896–917. doi:10.18632/oncotarget.387
- Diab, S.G., Clark, G.M., Osborne, C.K., Libby, A., Allred, D.C., Elledge, R.M., 1999. Tumor Characteristics and Clinical Outcome of Tubular and Mucinous Breast Carcinomas. *J. Clin. Oncol.* 17, 1442–1442. doi:10.1200/JCO.1999.17.5.1442
- Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J., Schreiber, R.D., 2002. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat. Immunol.* 3, 991–998. doi:10.1038/ni1102-991
- Dunn, G.P., Old, L.J., Schreiber, R.D., 2004. The Three Es of Cancer Immunoediting. *Annu. Rev. Immunol.* 22, 329–360. doi:10.1146/annurev.immunol.22.012703.104803
- Elenbaas, B., 2001. Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes Dev.* 15, 50–65. doi:10.1101/gad.828901
- Eroles, P., Bosch, A., Alejandro Pérez-Fidalgo, J., Lluch, A., 2012. Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. *Cancer Treat. Rev.* 38, 698–707. doi:10.1016/j.ctrv.2011.11.005
- Finak, G., Bertos, N., Pepin, F., Sadekova, S., Souleimanova, M., Zhao, H., Chen, H., Omeroglu, G., Meterissian, S., Omeroglu, A., Hallett, M., Park, M., 2008. Stromal gene expression predicts clinical outcome in breast cancer. *Nat. Med.* 14, 518–527. doi:10.1038/nm1764
- Fonseca, E.A.I., de Oliveira, M.A., Lobato, N. de S., Akamine, E.H., Colquhoun, A., de Carvalho, M.H.C., Zyngier, S.B., Fortes, Z.B., 2011. Metformin reduces the stimulatory effect of obesity on in vivo Walker-256 tumor development and increases the area of tumor necrosis. *Life Sci.* 88, 846–852. doi:10.1016/j.lfs.2011.03.005
- Geissmann, F., Jung, S., Littman, D.R., 2003. Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties. *Immunity* 19, 71–82. doi:10.1016/S1074-7613(03)00174-2
- Giannarelli, R., Aragona, M., Coppelli, A., Del Prato, S., 2003. Reducing insulin resistance with metformin: the evidence today. *Diabetes Metab.* 29, 6S28-6S35. doi:10.1016/S1262-3636(03)72785-2
- Gordon, S., Taylor, P.R., 2005. Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5, 953–964. doi:10.1038/nri1733
- Goodwin, P.J., Ligibel, J.A., Stambolic, V., 2009. Metformin in Breast Cancer: Time for Action. *J. Clin. Oncol.* 27, 3271–3273. doi:10.1200/JCO.2009.22.1630
- Guinebretière, J.M., Menet, E., Tardivon, A., Cherel, P., Vanel, D., 2005. Normal and pathological breast, the histological basis. *Eur. J. Radiol.* 54, 6–14. doi:10.1016/j.ejrad.2004.11.020

Chapitre 6. Références bibliographiques

Haugrud, A.B., Zhuang, Y., Coppock, J.D., Miskimins, W.K., 2014. Dichloroacetate enhances apoptotic cell death via oxidative damage and attenuates lactate production in metformin-treated breast cancer cells. *Breast Cancer Res. Treat.* 147, 539–550. doi:10.1007/s10549-014-3128-y

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of Cancer: The Next Generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hanahan, D., Weinberg, R.A., 2000. The Hallmarks of Cancer. *Cell* 100, 57–70. doi:10.1016/S0092-8674(00)81683-9

Harris, C.C., 1996. Structure and Function of the p53 Tumor Suppressor Gene: Clues for Rational Cancer Therapeutic Strategies. *JNCI J. Natl. Cancer Inst.* 88, 1442–1455. doi:10.1093/jnci/88.20.1442

Henouda, S., 2015. Breast Carcinoma in Younger Algerian Eastern Women: Epidemiological Profile in Series of 135 Cases. *Sci. Res.* 3, 198. doi:10.11648/j.sr.20150304.17

Isoda, K., 2005. Metformin Inhibits Proinflammatory Responses and Nuclear Factor- κ B in Human Vascular Wall Cells. *Arterioscler.Thromb.Vasc. Biol.* 26, 611–617. doi:10.1161/01.ATV.0000201938.78044.75

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. *CA. Cancer J. Clin.* 61, 69–90. doi:10.3322/caac.20107

Klein, G., 2012. Tumor resistance. *Oncolimmunology* 1, 1355–1359. doi:10.4161/onci.22194

Korkaya, H., Liu, S., Wicha, M.S., 2011. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. *J. Clin. Invest.* 121, 3804–3809. doi:10.1172/JCI57099

Kourelis, T.V., Siegel, R.D., 2012. Metformin and cancer: new applications for an old drug. *Med. Oncol.* 29, 1314–1327. doi:10.1007/s12032-011-9846-7

Lakshmi Narendra, B., Eshvendar Reddy, K., Shantikumar, S., Ramakrishna, S., 2013. Immune system: a double-edged sword in cancer. *Inflamm. Res.* 62, 823–834. doi:10.1007/s00011-013-0645-9

Lam, S.W., Jimenez, C.R., Boven, E., 2014. Breast cancer classification by proteomic technologies: Current state of knowledge. *Cancer Treat. Rev.* 40, 129–138. doi:10.1016/j.ctrv.2013.06.006

Leal, C.B., Schmitt, F.C., Bento, M.J., Maia, N.C., Lopes, C.S., 1995. Ductal carcinoma in situ of the breast. Histologic categorization and its relationship to ploidy and immunohistochemical expression of hormone receptors, p53, and c-erbB-2 protein. *Cancer* 75, 2123–2131. doi:10.1002/1097-0142(19950415)75:8<2123::AID-CNCR2820750815>3.0.CO;2-V

Liu, S., Ginestier, C., Ou, S.J., Clouthier, S.G., Patel, S.H., Monville, F., Korkaya, H., Heath, A., Dutcher, J., Kleer, C.G., Jung, Y., Dontu, G., Taichman, R., Wicha, M.S., 2011. Breast Cancer Stem Cells Are Regulated by Mesenchymal Stem Cells through Cytokine Networks. *Cancer Res.* 71, 614–624. doi:10.1158/0008-5472.CAN-10-0538

Chapitre 6. Références bibliographiques

- Lodén, M., Stighall, M., Nielsen, N.H., Roos, G., Emdin, S.O., Östlund, H., Landberg, G., 2002. The cyclin D1 high and cyclin E high subgroups of breast cancer: separate pathways in tumorigenesis based on pattern of genetic aberrations and inactivation of the pRb node. *Oncogene* 21, 4680–4690. doi:10.1038/sj.onc.1205578
- Mantovani, A., Bottazzi, B., Colotta, F., Sozzani, S., Ruco, L., 1992. The origin and function of tumor-associated macrophages. *Immunol. Today* 13, 265–270. doi:10.1016/0167-5699(92)90008-U
- Mittal, D., Gubin, M.M., Schreiber, R.D., Smyth, M.J., 2014. New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. *Curr. Opin. Immunol.* 27, 16–25. doi:10.1016/j.coi.2014.01.004
- Motoshima, H., Goldstein, B.J., Igata, M., Araki, E., 2006. AMPK and cell proliferation - AMPK as a therapeutic target for atherosclerosis and cancer: AMPK inhibits cell proliferation. *J. Physiol.* 574, 63–71. doi:10.1113/jphysiol.2006.108324
- Nass, S.J., Dickson, R.B., 1997. Defining a role for c-Myc in breast tumorigenesis. *Breast Cancer Res. Treat.* 44, 1–22. doi:10.1023/A:1005858611585
- Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., Weinberg, R.A., 2005. Stromal Fibroblasts Present in Invasive Human Breast Carcinomas Promote Tumor Growth and Angiogenesis through Elevated SDF-1/CXCL12 Secretion. *Cell* 121, 335–348. doi:10.1016/j.cell.2005.02.034
- Osborne, C., 2004. Oncogenes and Tumor Suppressor Genes in Breast Cancer: Potential Diagnostic and Therapeutic Applications. *The Oncologist* 9, 361–377. doi:10.1634/theoncologist.9-4-361
- Parihar, A., Eubank, T.D., Doseff, A.I., 2010. Monocytes and Macrophages Regulate Immunity through Dynamic Networks of Survival and Cell Death. *J. Innate Immun.* 2, 204–215. doi:10.1159/000296507
- Park, I., Kim, J., Kim, M., Bae, S.Y., Lee, S.K., Kil, W.H., Lee, J.E., Nam, S.J., 2013. Comparison of the Characteristics of Medullary Breast Carcinoma and Invasive Ductal Carcinoma. *J. Breast Cancer* 16, 417. doi:10.4048/jbc.2013.16.4.417
- Pollard, J.W., 2004. Opinion: Tumour-educated macrophages promote tumour progression and metastasis. *Nat. Rev. Cancer* 4, 71–78. doi:10.1038/nrc1256
- Prat, A., Parker, J.S., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J.I., He, X., Perou, C.M., 2010. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res.* 12. doi:10.1186/bcr2635
- Queiroz, E.A.I.F., Puukila, S., Eichler, R., Sampaio, S.C., Forsyth, H.L., Lees, S.J., Barbosa, A.M., Dekker, R.F.H., Fortes, Z.B., Khaper, N., 2014. Metformin Induces Apoptosis and Cell Cycle Arrest Mediated by Oxidative Stress, AMPK and FOXO3a in MCF-7 Breast Cancer Cells. *PLoS ONE* 9, e98207. doi:10.1371/journal.pone.0098207
- Rakha, E.A., Gandhi, N., Climent, F., van Deurzen, C.H.M., Haider, S.A., Dunk, L., Lee, A.H.S., Macmillan, D., Ellis, I.O., 2011. Encapsulated Papillary Carcinoma of the Breast: An

Chapitre 6. Références bibliographiques

- Invasive Tumor With Excellent Prognosis. *Am. J. Surg. Pathol.* 35, 1093–1103. doi:10.1097/PAS.0b013e31821b3f65
- Reed, A.E.M., Kutasovic, J.R., Lakhani, S.R., Simpson, P.T., 2015. Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. *Breast Cancer Res.* 17. doi:10.1186/s13058-015-0519-x
- Rena, G., Pearson, E., Sakamoto, K., 2013. Molecular mechanism of action of metformin: old or new insights? *Diabetologia* 56, 1898–1906. doi:10.1007/s00125-013-2991-0
- Ruggiero-Lopez, D., Lecomte, M., Moinet, G., Patereau, G., Lagarde, M., Wiernsperger, N., 1999. Reaction of metformin with dicarbonyl compounds. possible implication in the inhibition of advanced glycation end product formation. *Biochem. Pharmacol.* 58, 1765–1773. doi:10.1016/S0006-2952(99)00263-4
- Sherwood, L.M., Parris, E.E., Folkman, J., 1971. Tumor Angiogenesis: Therapeutic Implications. *N. Engl. J. Med.* 285, 1182–1186. doi:10.1056/NEJM197111182852108
- Shi, C., Pamer, E.G., 2011. Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.* 11, 762–774. doi:10.1038/nri3070
- SLAMON, J.D., GODOLPHIN, 1989. Studies of the HER-2/neu Proto-oncogene in Human Breast and Ovarian Cancer - ProQuest [WWW Document]. URL <http://search.proquest.com/openview/76eae886ef01a4c440c8e837d941833/1?pq-origsite=gscholar&cbl=1256> (accessed 5.28.17).
- Steeg, P.S., Zhou, Q., 1998. Cyclins and breast cancer. *Breast Cancer Res. Treat.* 52, 17–28. doi:10.1023/A:1006102916060
- Strauss, L., Bergmann, C., Szczepanski, M., Gooding, W., Johnson, J.T., Whiteside, T.L., 2007. A Unique Subset of CD4+CD25highFoxp3+ T Cells Secreting Interleukin-10 and Transforming Growth Factor-1 Mediates Suppression in the Tumor Microenvironment. *Clin. Cancer Res.* 13, 4345–4354. doi:10.1158/1078-0432.CCR-07-0472
- Swann, J.B., Smyth, M.J., 2007. Immune surveillance of tumors. *J. Clin. Invest.* 117, 1137–1146. doi:10.1172/JCI31405
- Szewczyk, M., Richter, C., Briese, V., Richter, D.-U., 2012. A retrospective in vitro study of the impact of anti-diabetics and cardioselective pharmaceuticals on breast cancer. *Anticancer Res.* 32, 2133–2138.
- Talmadge, J.E., Fidler, I.J., 2010. AACR Centennial Series: The Biology of Cancer Metastasis: Historical Perspective. *Cancer Res.* 70, 5649–5669. doi:10.1158/0008-5472.CAN-10-1040
- Vence, L., Palucka, A.K., Fay, J.W., Ito, T., Liu, Y.-J., Banchereau, J., Ueno, H., 2007. Circulating tumor antigen-specific regulatory T cells in patients with metastatic melanoma. *Proc. Natl. Acad. Sci.* 104, 20884–20889. doi:10.1073/pnas.0710557105
- Walsh, M.M., Bleiweiss, I.J., 2001. Invasive micropapillary carcinoma of the breast: Eighty cases of an underrecognized entity. *Hum. Pathol.* 32, 583–589. doi:10.1053/hupa.2001.24988

Chapitre 6. Références bibliographiques

Whitelaw, D.M., 1966. The intravascular lifespan of monocytes. *Blood* 28, 455–464.

Whiteside, T.L., 2008. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 27, 5904–5912. doi:10.1038/onc.2008.271

Wiktor-Jedrzejczak, W., Gordon, S., 1996. Cytokine regulation of the macrophage (M phi) system studied using the colony stimulating factor-1-deficient op/op mouse. *Physiol. Rev.* 76, 927–947.

Witton, C.J., Reeves, J.R., Going, J.J., Cooke, T.G., Bartlett, J.M., 2003. Expression of the HER1–4 family of receptor tyrosine kinases in breast cancer. *J. Pathol.* 200, 290–297. doi:10.1002/path.1370

Zordoky, B.N.M., Bark, D., Soltys, C.L., Sung, M.M., Dyck, J.R.B., 2014. The anti-proliferative effect of metformin in triple-negative MDA-MB-231 breast cancer cells is highly dependent on glucose concentration: Implications for cancer therapy and prevention. *Biochim.Biophys.Acta BBA - Gen. Subj.* 1840, 1943–1957. doi:10.1016/j.bbagen.2014.01.023